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EVOLUTION AND SPECIATION IN MOSSES*

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As a footnote to the title I have chosen, I should say that the word "mosses" has been used intentionally here in one of its widest and oldest senses, to include peat mosses, hepatics, and hornworts, as well as the true mosses (Hedwig, 1784; Campbell, 1895). More technically, these plants are all called bryophytes, although the formerly more comprehensive category, Bryophyta, has recently been given a much restricted meaning (Bold, 1956), about which more will be said later.

A consistent historical trend in the classification of the Plant Kingdom has been the multiplication of divisions, through the gradual recognition of fundamental differences between groups formerly considered to be rather closely related. From the classification of the Plant Kingdom proposed by Eichler in 1883, with four divisions, widely accepted a generation ago, we now have progressed to 24 divisions in one of the more recent classifications, proposed by Bold (1956, 1957). Among other innovations, Bold has modified the classical concept of the division Bryophyta, which contained both mosses and hepatics (Smith, 1955), by raising each of the two major former classes, Hepaticae and Musci, to the rank of division, the Hepatophyta and the Bryophyta. I view my friend Harold Bold's treatment with mixed emotions.

As a teacher, I cannot help but deplore the restriction of the time-honored term, "Bryophyta," to mosses alone, because of the confusion that is certain to result. As a professional bryologist, on the other hand, I can go much further than Bold has, and with equal justice raise other highly distinctive groups of bryophytes to divisional rank. The peat-mosses, or Sphagna, and the hornworts or Anthocerotes are as distinctive in their own ways as are the true mosses and hepatics. On the other hand, in a search for differences, and with strong emphasis placed on distinctions, we stand in more than slight danger of overlooking basic similarities and relationships. With the gradual but continued devaluation or inflation of the rank of division, we need some kind of new category, perhaps a super-division or

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sub-sub-Kingdom, to bring the bryophytes back together again, no matter how widely and how deeply we may split them first.

A brief consideration of classification is valid here, I feel, because one purpose of classification is to represent, at a sort of cross-sectional level, our best ideas on the phylogeny of a group and the interrelationships within the group.

With this introduction, I shall elaborate somewhat on the several distinctive groups of "mosses," partly for orientation, and partly to establish a background from which to develop more general ideas.

I. *The hornworts or Anthocerotes* form a very isolated, natural, and distinctive group of plants (Howe, 1899; Bartlett, 1928). Several peculiar or unique features characterize them: (1) The sporophyte is the only one among "mosses" that continues growth in length, forming new spore mother cells at the base while releasing mature spores at the apex, through the presence of a meristematic zone just above the foot. (2) The very large chloroplasts, sometimes only one to a cell, and the usual presence of a pyrenoid, are unique features in bryophytes. (3) The immersed antheridia and archegonia are quite unlike those of other "mosses." (4) The presence of a columella around which the spores are produced, the presence of highly developed stomata and photosynthetic tissue in the walls of the capsule, the relative rarity of symbiotic fungi and especially the distinctive chromosome number, $n = 5$ (Mehra and Hundoo, 1953), among a whole constellation of other features, clearly demarcate the Anthocerotes from the hepatics. So much has been written, proven, speculated, and theorized about this group that I need say no more about it, except to state that it abundantly merits promotion to the category of Division, with the new name, Anthocerophyta.

II. *The true hepatics or liverworts*—Hepatophyta of Bold (1956)—consist of several diverse evolutionary lines whose high degree of specialization has led to more detailed morphological studies than in any other group of bryophytes. The vegetative plants vary from extremely simple to highly specialized cell masses or thalli, or consist of leafy stems, with almost all intermediate conditions. The sporophytes likewise present a wide range from a simple spherical case full of spores to more complex structures consisting of an attachment organ or foot, a stalk, sometimes very long, and a spore case with specialized devices for the release of spores. Much has been written on the evolution of hepatics, as reasoned from their present-day morphology, especially by Bower (1908, 1935), by Campbell (1895, 1940), and by Goebel (1905). Goebel, and more recently Evans (1939) and Mehra (1957), saw clear lines of reduction in hepatics, and presented evidence for the development of thallose forms from leafy stems. In contrast to the diversity of form and structure among hepatics, taken as a whole, we find a remarkable uniformity in the presence of elaters among the spores, and, only in the last few years, in the chromosome numbers. By far the great majority of hepatics so far investigated has the chromosome number, $n = 9$. In a very few genera we find the number, $n = 10$, and in somewhat

more genera, $n = 8$. In a few species the chromosome number is complicated by the sex-determining mechanism, so that the different sexes will have different numbers. As indicated by studies already published, polyploidy is remarkably rare among hepatics, in comparison with its incidence in true mosses.

III. *In peat mosses or "Sphagnobrya"*—which all belong to a single genus, Sphagnum—leafy plants arise from buds formed around the margin of a flat thallus. The fascicled branches, the complicated stem structure, the remarkable leaf consisting of a mesh of small green cells fencing in large bubble-like dead cells, the total lack both of rhizoids and of special means for vegetative reproduction are unique features among bryophytes. Other noteworthy characters are the long-pedicelled spherical antheridia, more like those of hepatics than of mosses, and the development of a pseudopodium at the apex of the vegetative plant that carries the sessile spore capsule aloft. Lewis E. Anderson tells me (personal communication) that under water this pseudopodium may become several inches long. The sporophyte differs from that of true mosses in several fundamental ways, including its embryogeny. In summary, I agree with Chalaud (1946) that the Sphagna are completely separate from other bryophytes and should be so recognized, formally. Following new trends then, the subclass Sphagnobrya (Smith, 1955, p. 96) should be promoted to the category of a Division.

By one of those curious coincidences so often found in scientific investigation, three separate studies on the cytology of Sphagnum were published recently by different individuals in different countries: in the United States (Bryan, 1955); in Denmark (Holmen, 1955); and in Finland (Sorsa, 1955, 1956). The remarkably constant chromosome number found in Sphagnum, $n = 19$, varied only by polyploidy, adds still further evidence for the independence of the group.

Sorsa (1956) offers some thought-provoking observations, as follows: "The moss order Sphagnales is extremely uniform with regard to its appearance. Cytological investigation indicates that the chromosome number is also very uniform, varying only as regards the degree of polyploidy. On the basis of observations made up to now, it seems that the chromosome number of the diploid sporophytes is 19. Eighteen of these form nine bivalents in meiosis and one is a univalent chromosome. This signifies that in the following haplophase there are always 9 chromosomes in half of the gametophytes and 10 in the others. In order that these chromosome numbers can be kept constant during alternation of generations, it is obvious that the gametes of different sex must always have exactly the chromosome numbers 9 and 10, which in fertilization come into the same sporophyte and in meiosis separate again." Another possibility, of course, is that the cluster of minute chromatic bodies that Sorsa interprets as a univalent chromosome, may actually be distributed evenly at meiosis. It would be interesting to have data on somatic counts of the different sexes, and to study meiosis in the polyploid species.

Whether we follow Sorsa's idea that the basic *diploid* number is 19, or Bryan and Holmen in believing that the basic *haploid* number is 19, plus of course the several minute "accessory chromosomes," we still find a remarkable stability in chromosome number, a monotony broken only by occasional polyploidy. Moreover, if one accepts the chromosome numbers postulated by Sorsa, he is immediately struck by the remarkable parallelism to chromosome numbers in Hepaticae, $n = 9$ and 10.

One final conclusion is obvious—further cytological study of Sphagnum will be fruitful indeed.

IV. *True mosses or Bryophyta* in the sense of Bold (1956) comprise the largest and most complex, as well as the most obviously polyphyletic group of "mosses" in the broadest interpretation of the word. The vegetative plants, without exception, are leafy and usually have stems, which may reach a length of several feet. The sporpohytes vary from simple cases full of spores, attached to the vegetative plant by a simple absorbing organ, to relatively complex structures consisting of a highly developed absorbing organ, a long stalk with complex conducting tissues, and a highly specialized, highly photosynthetic capsule, with complex structures and mechanisms for the release and dispersal of spores. The usual occurrence of stomata on the capsule walls, the germination of the spores to form protonemata, the invariably leafy stems, and many other common features give an over-all appearance of homogeneity to the group that disappears only when careful attention is given to the component families, orders—and now, classes. Overwhelmingly distinct and even unique modifications are to be found in groups not normally thought of as being remarkable. The superficial lack of morphological diversity of the degree found in hepatics has militated against detailed research on the morphology of true mosses. The genus Andreaea, classically considered as the center of a highly modified order of mosses, holds a very uncertain phylogenetic position. It possesses several unique features of structure, habitat and distribution that might indicate a systematic position as separate and distinct as that of Sphagnum. In fact, the presence on the vegetative plant of a pseudopodium that lifts up the sessile sporophyte to the wind is distinctly reminiscent of Sphagnum. However, the very different structure of both the vegetative plant, and the spore-producing plant, especially in the unique mechanism for capsule dehiscence, as well as the wholly different habitat, set the present subclass Andreaobrya (Smith, 1955) far apart from Sphagnum and from all other bryophytes. The chromosome number, $n = 10$ (Bryan, personal communication) is non-controversial; it agrees with that reported for both hepatics and mosses. These plants now restricted to the most inhospitable parts of the world—barren acid rocks at high altitudes and high latitudes—are among the few terrestrial plants so far discovered on the antarctic continent. If one can use the reasoning of the ethnologist, these unique mosses must surely comprise a group that is both ancient and primitive.

The genus Archidium is again the center of a distinctive group of mosses that, although diminutive in stature, present characters unique among

mosses, especially the small and determinate number of spores of extraordinarily large size, and the complete lack of a columella.

Serious study of the mosses will make necessary the reclassification, dismemberment and regrouping of many present families and orders. I think especially of the Tetraphidales, the Polytrichales, the Hookeriales, the Splachnales, and many more that represent present-day survivors of widely divergent phylogenetic lines, some of which may merit a much higher rank, in terms of category, than they now have.

One of the most interesting aspects of mosses, taken as a whole, is the variable chromosome number, ranging from $n = 5$ to $n = 66$, much as in flowering plants, a situation entirely different from the uniformly, almost monotonously, stable number reported in hepatics, Anthocerophyta, and in Sphagnum, and the high degree of polyploidy (see recent review by Wylie, 1957).

So far, I have attempted to identify the four unique and easily separable groups of byrophytes primarily on the basis of their differences. However, the need for synthesis is as rampant among botanists as it is among other biologists, as we see from the invention and wide use of so many relatively artificial, unofficial and sometimes overlapping but not synonymous categories, as Archegoniates, Embryophyta, vascular plants, Cryptogams, Conifers, flowering plants, thallophytes, etc., which have served us well as convenient teaching devices. Since the name Bryophyta has now been preempted in a more restricted sense by Bold (1956) for the former class, Musci, and since two further divisions are proposed here, we need some taxon to receive these new divisions, and to indicate that they all belong to the same polyphyletic constellation, some sort of super-super division. To save trouble here, and for convenience only, I have simply called the members of this natural group "mosses." It should be emphasized that certain fundamental and underlying similarities and relationships do exist among all groups of bryophytes, no matter how far apart they may appear to be, in our cross-sectional view today, as the evolutionary apices of widely divergent phylogenetic lines. They *all* have biflagellate sperms, multicellular sex organs with sterile one-layered coverings or jackets, similar sporophytes permanently attached to the gametophytic plant and dependent upon it perhaps for part of its moisture, although it is normally green and can produce all or most of its own carbohydrates (Studhalter, 1938; Bold, 1938, 1940, 1948, 1952; Freeland, 1957). The life history and pattern of alternation of generations is identical in all these groups, a feature that should carry considerable weight, at least in philosophical discussions, until we find other criteria that may tend to subjugate the importance of life histories. I suggest that we recognize in an informal manner, a new group, the "Mosses and Moss Allies," just as a generation and more ago, we recognized the "Ferns and Fern Allies," grouped together then under the Pteridophyta—and just as Goebel (1905) continued to use "Bryophyta" for both liverworts and mosses, even after pointing out that no transition forms connect them. By the same token and the same example, we may in the coming

generation discover that our presently considered groups, diverse and uneasily anchored together today in this "Superbryophyta," may be widely separated phylogenetically and taxonomically, and actually form centers of other constellations of classification.

PHYLOGENY

Bradley Moore Davis came to Stanford from Indiana University with Douglas Houghton Campbell as his student, and was a member of our first graduating class in 1892. As one of my first botany professors at the University of Michigan, he firmly convinced me that the sporophyte of mosses and hepatics had evolved gradually through the intercalation of an increasing number of mitotic divisions between the fertilized egg or first diploid cell, and meiosis and spore production. While I was a student, Campbell's sonorous phrase (adapted from Bower), that the sporophyte developed phylogenetically through "the progressive sterilization of potentially sporogenous tissue," seemed to me apt and logical. Somewhat later, however, I came to understand the persuasive arguments put forth for an opposite point of view, that alternation of generations in higher plants could possibly represent a much more advanced aspect of the relatively simple isomorphic haplont-diplont situation still found in some green algae, as Ulva (Papenfuss, 1957), where the sexual and asexual plants are identical but separate. If we follow this theory, which has found increasing support (Fritsch, 1945; Bold, 1940 and 1948), we must assume that the earliest bryophyte-like plants must have had sporophytes entirely to nearly independent from the gametophytes and rather similar to each other in appearance (Zimmermann, 1932, 1955). Two of the most interesting bits of evidence in this direction are also most commonly overlooked; the young sporophytes of all "mosses" are green and from somewhat to highly photosynthetic; moreover, the young sporophyte of true mosses regenerates protonema, presumably a gametophytic structure. The great morphologists (Bower, Campbell, and Goebel) worked with materials preserved in alcohol and other solutions that removed chlorophyll. Consequently the statement so frequent in elementary and even in advanced texts that the sporophyte of bryophytes is *completely dependent on the gametophyte* is quite false (Bold, 1938; Studhalter, 1938). Anyone who studies "mosses" in the field knows that the sporophytes are green up to the moment that the spores begin to mature; and that some of them must even provide nourishment to the vegetative plant. Consequently, it is interesting to see the casual notes published from time to time by morphologists who have just "discovered" this fact. Campbell's discovery (1917) of an Anthoceros sporophyte that had almost become independent is another factor here, as is the odd and nearly self-supporting capsule of Buxbaumia, and other mosses whose vegetative plant is reduced nearly to the point of invisibility.

During the past decade a good deal of attention has been given to the possible phylogenetic source of the bryophytes. Margaret Fulford (1948) has provided us with an excellent review covering the hepatics. Of the

several theories that have been postulated, some of the more important follow:

1. The classical idea that bryophytes arose directly from some now extinct group of algae, postulated by Davis (1903) and many others. Unfortunately, Davis used as a theoretical prototype of the multicellular sex organs of bryophytes the plurilocular gametangia of Ectocarpus, which we now know to be far outside the genetic and evolutionary stream of the Chlorophyta. On physiological and biochemical grounds the chlorophyta include the green algae, the bryophytes, the ferns and their allies, and the seed plants. However, in view of the enormous length during which plants have evolved, at least 1500 million years, and the innate tendency of plants to experiment under the urgent and changing requirements of new environmental niches, as well as whole new environmental circumstances, almost anything could have happened, and yet have left very little trace behind.

2. The discovery of extremely simple pteridophytes in lower Devonian and late Silurian deposits has resulted in their comparison with spore-producing structures in the Anthocerotes, with strongly suggestive results (Kashyap, 1919). The remarkable similarity in the spore cases has led to a surge of speculation that bryophytes, and especially Anthocerotes, are only reduction forms derived from different types of primitive (or simple) pteridophytes. Haskell (1949) accepts this point of view without question. He concludes his paper with the remark (p. 57) that: "Although the Bryophyta may be modifications of Psilophytales and form a group so modified as to end blindly, their position with the Pteridophyta is unchanged." Christiansen in a recent paper (1954) likewise accepts fully the idea that the Bryophyta are derived from the Pteridophyta, and gives three alternative sources, as follows: (1) From Pteridophyta with leaves on the stem of the gametophyte as well as that of the sporophyte, (2) from such leafless Pteridophyta as the Rhyniaceae, and (3) the bryophytes are polyphyletic, derived from different types of Pteridophyta, some leaf-bearing, others not.

3. I agree with Christensen's third alternative, to the extent that bryophytes are certainly polyphyletic, but not that they have been derived from pteridophytes as such. In my opinion both groups must have come from a common and more primitive stock, long since extinct, which itself may have been polyphyletic. To me, the bryophytes are an ancient phylogenetic line or series of lines that have come to a dead-end, evolutionarily speaking. Although they have developed some remarkable adaptations to their terrestrial habitat (Bower, 1908), the latter are the adaptations of primitive plants and not of advanced ones. For example, lacking a cutinized epidermis or other protective tissue, the delicate leaves of "mosses," almost always only a single layer of cells in thickness, dry out in the full sunshine of summer to such an extent that they may be easily rubbed to powder between one's hands. Undamaged plants resume growth almost immediately when moistened by dew, fog or rain. In plants reduced to powder, however, each single vegetative cell still has the totipotent property of regenerating a whole new plant or eventually a whole new colony of new plants. This

vital capacity as well as the clustered, almost colonial, habit of the vegetative plants, reminds us more of lower plants than of higher ones.

In our reflections on the phylogeny of mosses and their relatives, we can be always helped by the thought-provoking warning given by Goebel (1905, p. 7) more than 50 years ago:

> Between Hepaticae and Musci there are no transition-forms, as there are none between Bryophyta and Pteridophyta; and as there never were such transitions their absence is not caused by their having died out. If the development proceeded from very simple nearly related forms in definite and divergent directions we ought always to find a partial correspondence only in the simplest forms, and as a matter of fact we find these. All of the speculations upon the relationship between the Hepaticae and Musci, Bryophyta and Pteridophyta, and other groups, which are based upon the highly developed Archegoniatae are therefore products of fancy; they spring from the tendency of our imagination to assume connexions even where they are not directly proved, but they have no sufficient support in the facts of experience, and their sole value lies in the new points of discussion they create.

As an interesting example of the lengths to which imaginative speculations may go, I cite the highly original idea proposed by Appl (1941) that the sudden appearance of a highly differentiated angiosperm flora in geological history may be explained by the rapid evolution of mosses into flowering plants.

TIME OF ORIGIN

The time in the geological scale at which the first bryophyte evolved is still one of the real and classical botanical mysteries. As long ago as 1911, D. H. Scott recognized the great puzzle posed by the question of origin of the bryophytes. The relationship of the sexual vegetative plant to Algae on the one hand, and the obvious relationship of the sporophyte, on the other hand, to higher plants through the possession of typical stomata, as well developed as in vascular plants, in most true mosses and in Anthocerotes is indeed a baffling situation. The fossil liverworts found in the upper Carboniferous deposits of England are so similar in structure and appearance to modern species that their discoverer, Walton (1925), was not at first satisfied that they had come from the Paleozoic shales he was studying. Consequently, he continued making further preparations until he was convinced. Walton later (1928) postulated the existence of Paleozoic mosses, although on evidence not nearly as conclusive as for the hepatics. Other groups of bryophytes that are now extinct once existed in abundance, a view supported by the monumental study of Naiadita by Harris (1938, 1939). It is just as certain that some groups of living bryophytes are extremely ancient, as shown by their geographical distribution, their morphological structure, and the intuitive feelings of bryologists (Steere, 1937). The apparent lack of fossil evidence for the presence of bryophytes before Carboniferous times has been evaluated by Haskell (1949), as follows: "The fossil record also shows that plants with cuticle, stomata or lignified vascular tissue occurred in the Silurian. This suggests two possibilities concerning the absence of Bryophytes earlier than the Carboniferous.

Firstly, the records are incomplete and Bryophyta may yet be discovered in Silurian rocks, providing physical conditions have not destroyed them, which is unlikely since other delicate plants have been found. The other is that *they did not occur as they were not yet evolved*, that is, they arose at least after the Devonian flora."

My own belief is that bryophytes arose very early, but because of their small size, their delicate tissues, their general lack of cutin, and their simple structures, they merely have left a very poor fossil record (Steere, 1946). Even in much more recent deposits, and in terms of thousands rather than of millions of years, when we reconstruct floras whose consistency we can predict with a high degree of probability if not certainty, many species of mosses and hepatics are missing from peats and soils buried under Pleistocene deposits that we know must have been there, in terms of other species present. In other words, the chemistry of some bryophytes has guaranteed their preservation, but in others it has guaranteed their disappearance (Steere, 1942). The fact that no fossil bryophyte found so far, no matter how ancient, is more primitive or less specialized than our modern forms, I hold to be highly significant in evaluating the age of the group. The report of spores with trilete dehiscence, of the sort found today in some bryophytes and pteridophytes, in the Lower Cambrian of the eastern Baltic (Noumova, 1949) suggests dramatically that terrestrial plants must have originated in pre-Cambrian times, very much earlier than hitherto suspected.

The discovery of primitive algae and fungi in Pre-Cambrian cherts in southern Ontario (Tyler and Barghoorn, 1954), estimated to average 1300 million years old, presents a time dimension for us to consider seriously in our speculations on the phylogeny of bryophytes, and may help correct the casual acceptance of the opinion that bryophytes are of recent development, and that recent forms must be considered to be closely related.

The remarkable assortment of delicate tissues recovered by Mamay (unpublished) from maceration of marine deposits of Carboniferous age, from Oklahoma, makes one wonder what might turn up from similar treatment of richly fossiliferous freshwater deposits of Devonian and earlier ages. We will continue to live in the ever increasing hope, or even with the expectation, that further discoveries and better techniques—as well as increasing interest in paleobotany—may yet turn up our "missing link."

SPECIATION

Now, after having disposed of the broader aspects of evolution, in general and philosophical terms, let's look at the smaller, day-by-day aspects of speciation, about which we perhaps know a bit more—or perhaps a bit less.

I have used "speciation" here possibly in too loose a sense, since I shall discuss several specific evolutionary trends that have resulted in interesting specializations and modifications. The exploitation of some one highly evolved morphological feature in every possible way by the elaboration and variation of the theme as in music, has resulted in large

constellations of species and genera, obviously related, yet just as obviously distinct, e.g., the development of the double midrib in the leaf and of the characteristic calyptra in the tropical moss family, Hookeriaceae; the elaboration of the water sac in the leaves of the enormous hepatic family Lejeuneaceae; the habitat specialization and the odd capsule structure in the moss family Splachnaceae; the remarkable homogeneity in structure of the peat mosses, which nevertheless present several clear evolutionary lines.

Mosses and their allies are unique among plants because the green vegetative plant is the haploid sexual generation, and the diploid spore-producing structure, although green throughout its development, is attached to it permently. The green sexual plants grow rapidly and may form extensive and often conspicuous masses of vegetation. Moreover, most species have developed some means of vegetative reproduction, so that a large colony of any one species may be a single clone. Each sporophyte, however, results from a separate fertilization. Although the clonal nature of the sexual plants may tend to reduce the amount of outbreeding, it cannot prevent it, since free-swimming sperms may be splashed or washed in from some distance. As a consequence of the different nature and function of the haploid sexual plant and the diploid asexual plant that "roosts" on it at least while it is producing spores, these two plants have evolved at different rates and in different directions. To the botanist who can read one evolutionary story in the sexual plant and quite another in the sporophyte, mosses are quite literally "double-headed monsters." In the hepatics and in peat mosses, the sporangium is extremely conservative and rarely shows distinctive differences between closely related species. In the true mosses, on the other hand, one occasionally finds clear-cut and consistent differences between species in their spore capsules, occasionally in genera in which the green plant may be highly conservative or unspecialized.

Several examples of some unusual specializations may be of interest here. Because of time limitations tonight and because I am most familiar with the true mosses, most of my examples will be chosen from them. Lorch (1931) has given us an excellent treatment of the structure of mosses, and the enormous range of variation to be found in every organ.

The genus Schistostega is highly modified for living under greatly reduced light conditions in caves and holes, where in the struggle for existence, it finds little competition from other plants. Although it produces leafy plants and occasional sporophytes, the bulk of the vegetation of this plant consists of sprays of filamentous protonemata, especially where new areas are being colonized. The individual cells of the protonema are parabolic and aim themselves automatically at the light source. The chloroplasts arrange themselves at the bottom of the cell at the exact focus of the light, which is so concentrated on them that they glow with a soft greenish-yellow fluorescence. The light reflected from these microscopic cells, en masse, is distinctly visible to the human eye, when viewed from the direction of the light source—and if not obstructed. When one walks

up to the softly glowing moss colony, however, he either cuts off the light, or changes his angle of vision, so that the glow disappears. This so-called "luminous moss" is almost certainly the source of medieval stories of "fairy gold" in caves.

Although the alga-like protonema would seem to be the simplest and most primitive aspect of vegetative mosses, it nonetheless presents many interesting biological problems. Especially interesting is the orderly production of buds that become leafy stems, along the filamentous protonema that consists only of a single file of cells joined end to end. In many mosses the protonemal stage is persistent, highly photosynthetic, and produces only scattered leafy plants. The protonemal stage is easily grown in pure culture and might provide an exceptionally facile experimental material for a study of the hormonal situation that induces a filamentous structure to produce something quite different.

One of the strangest modifications in mosses, and one which has arisen independently in several unrelated groups, is the dwarfing of male plants to such an extent that they are microscopic, and epiphytic on the stem or even the leaves of female plants several inches long. Although Ernst-Schwarzenbach reported heterospory in Macromitrium (1939, 1944), it was on a purely statistical basis. Spore measurements in other species in which the male plants are microscopic show no statistical heterospory; the measurements fall along the normal hay-stack curve. Two approaches to this interesting problem are urgently needed: a cytological study of the sex-determining mechanism, and a cultural study of the male plants to see if their growth has been inhibited by hormones from the female plant upon which they develop, or whether they are genetically dwarfed. The use of gibberellins might yield some interesting data in this connection.

Unlike nearly all other mosses, Buxbaumia possesses a microscopic and short-lived green sexual plant. The oddly shaped sporophyte is highly photosynthetic from its inception, and moreover grows in soils rich in humus or directly on rotten wood, which suggests a partially saprophytic means of nutrition. There is also some possibility of a symbiotic relationship with a fungus or alga in the soil, from the uniform appearance of the habitat, although the nutrition of this unique moss has not been studied in modern terms.

One of the most interesting families of mosses, in the evolution of both nutritional and morphological modifications, is the Splachnaceae, which, in general, inhabits colder areas at high altitudes and high latitudes. However, in these areas where the soil tends to be sterile, members of the several genera of Splachnaceae grow on the droppings of animals and other highly nitrogenous materials, as the carcasses and bones of animals, and windrows of insect exuviae on beaches. Near the tip of Point Barrow, Alaska, where for centuries the Eskimos have been butchering seals, walrus, and whales, the ground is soaked with the body fluids of these animals—and supports an extraordinary population of splachnaceous mosses. The capsules of members of the Splachnaceae, are among the most highly photosynthetic in mosses, and especially in the genus Splachnum, highly

modified and colored structures occur below the capsule which are reported to attract insects (Erlanson, 1935), who in turn carry the spores to other favorable habitats. So far as I know, these are the only mosses whose capsules have specific odors. The capsule of Tetraplodon has the strong sharp odor of an acetic ester. *Splachnum ovatum* smells strongly of lactic acid, and *S. luteum* is reported to have the odor of a butyl compound (William S. Benninghoff, personal communication). A correlation that may have some significance is that the more highly modified and specialized species, morphologically, also have the odor of the more complex organic compound. The biochemical evolution in Splachnum has not been studied, and would seem to promise a fertile field for investigation.

Important genetical studies made and summarized by Wettstein (1923-4, 1928, 1942) on mosses and by Allen (1945) and Burgeff (1943) on hepatics, have shed a good deal of light on patterns of inheritance in these small plants, as well as on the results of mutation and of hybridization. However, the surface is barely scratched in these areas, and an enormous amount of work still remains to be done.

Mosses and their allies have no true roots, and are therefore much more sensitive to and restricted by the rigors of their superficial environment. The most important single factor in the environment to bryophytes is moisture, and these small plants are subject to many sudden morphological modifications from equally sudden changes in water supply. Other environmental factors are also important in their bearing and impact on morphological structure. The recent and important studies by Margaret Fulford (1954) and her colleagues (Diller et al., 1955), and the culture in bacteria-free colonies by my student Grace Iverson (unpublished data) on hepatics indicates clearly how variable these plants are, yet how, finally, we may test the stature and dignity of species through culture under identical environmental conditions.

POLYPLOIDY

It is appropriate to recall here that the first polyploid series known in plants was created artificially in mosses through apospory, which involved the first experimental study of the mechanisms of speciation in plants. Pringsheim (1878) discovered that the young sporophytes of several species of true mosses would regenerate diploid gametophytic tissues, but his discovery was so far ahead of its time that its importance was not then properly appreciated. With the realization by Strasburger (1894) of the cytological differentiation of sporophyte and gametophyte and of the alternation of a haploid sexual generation with a diploid asexual generation, the significance of apospory was finally understood. The brilliantly conceived and executed researches of the Marchals resulted in the experimental production of a tetraploid race ("var. *bivalens*") of *Bryum capillare* (Marchals, 1911) and of tetraploid and octaploid ("var. *bivalens*" and "var. *quadrivalens*" of *Amblystegium serpens* (Marchals, 1911, 1912)). Wettstein and others, in more recent investigations (1923-24, 1928), succeeded in producing auto-

ploid and amphiploid races in several mosses, including *Funaria hygrometrica* ($n = 18$, $n = 28$, $n = 56$), *Physcomitrium piriforme* ($n = 18$, $n = 36$, $n = 72$), *Bryum caespiticium* ($n = 10$, $n = 20$), and *Ambylstegium serpens* ($n = 12$, $n = 24$). The discovery of polyploids in natural populations was much delayed, however, largely because of technical difficulties in counting chromosomes. The first report of polyploidy in mosses, in nature, seems to be that of Heitz (1926) of a tetraploid (in terms of sporophyte) race of *Atrichum undulatum* with the chromosome number of "14-16," and his subsequent report (1928) of a hexaploid race with the number "(20)21(22)." Later, Heitz (1942) and Lowry (1948) pointed out the existence in nature of paired species, one diploid and the other tetraploid, in the genus Mnium. They further showed a significant relationship between such paired species, that the diploid species are heterothallic and that the tetraploid species are homothallic, indicating a strong possibility that the tetraploid species arose through sporophytic regeneration or apospory. Of course, there is a possibility also that the tetraploid races may occasionally arise from unreduced, diploid spores, which are known to occur in mosses. However, diploid spores are normally formed by suppression of the second meiotic division, *after* the segregation of homologous chromosomes, and consequently would not be expected to produce homothallic tetraploid plants, if the parent plant was heterothallic. This fact lends force to Heitz's hypothesis, supported by Lowry's results, that natural apospory has been effective in producing tetraploid races or species of true mosses. Since these paired species, one diploid and one tetraploid, have not yet been reproduced in the laboratory through the experimental induction of apospory under controlled experimental conditions, we still do not know whether in nature they arose long ago and have undergone further evolution to reach their present clear-cut specific isolation, or whether they have been produced repeatedly, de novo, up to the present time. The latter possibility presents a striking and unique means of polyphyletic speciation, by which all populations comprising a tetraploid or polyploid species might not be genetically related, except in terms of the parent diploid species.

As has been indicated earlier, the true mosses are the only group of bryophytes with a wide range in chromosome number, in sharp contrast to the other groups which have so little variation in number that the results seem monotonous.

The question arises occasionally whether or not the species of lower green plants are comparable or coordinate with the species of higher plants, a problem that has received some serious consideration by botanists. The cytological behavior of bryophytes, especially of true mosses, appears to parallel that of flowering plants very closely. This parallelism is shown especially well by the existence in true mosses of highly polyploid species (Steere, Anderson, and Bryan, 1954; Steere, 1955), and aneuploid races, the multiple association of chromosomes during the first and second meiotic divisions, the precocious disjunction of bivalent chromosomes and of chromatids, the presence of minute or "accessory" chromosomes, and by the

early discovery of sex chromosomes in hepatics (Allen, 1917). Finally, it seems safe to conclude that the species of mosses, and apparently of bryophytes in general, in spite of their relatively simple morphology, present the same kind and degree of complexity as those of higher plants, and that speciation within natural populations of mosses is governed by the same patterns of cytological behavior (Steere, Anderson, and Bryan, 1954).

My summary and conclusion is very brief. I shall only point out once more that mosses and their allies present some remarkably promising but relatively untouched materials for experimental research on basic and fundamental problems of evolution and speciation in plants.

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STUDIES ON SEXUAL SELECTION IN MICE

I. Reproductive Competition between Albino and Black-Agouti Males

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In his consideration of the forces involved in evolution, Darwin (1887) gave considerable emphasis to the phenomenon he called "sexual selection." He defined it as "...the advantage which certain individuals have over others of the same sex and species solely in respect to reproduction." Darwin felt that sexual selection could result either from a preference shown by the female for some of the competing males or from an interaction between competing males in which one male excluded the other from mating. He found competitive mating systems to be widespread, due in many cases to an excess of males over females in the population. The competitive aspect was further accentuated, in some species, by the existence of polygamy. Darwin realized that any individual variation that resulted in a higher reproductive rate would be transmitted to offspring and hence be important in evolution. He even considered the possibility that factors acting in sexual selection might have no function in the general struggle for existence and he drew a distinction between sexual selection and natural selection, although he fully realized that in many cases it is impossible to distinguish between the effect of these two phenomena.

Since Darwin's time, sexual selection has been studied in many organisms. In a large number of cases it has been shown that certain males tend to exclude other males from the reproductive phase of group life (Alverdes, 1935; King, 1929; Uhrich, 1938). However the possibility that sexual selection operates in mice has not been well studied. The present paper is the first report on a series of experiments designed to investigate various aspects of sexual selection in mice. These will include a study of the genetic basis of competitive mating behavior and an examination of its possible role in the maintenance of certain alleles (*t*) in wild mouse populations.

MATERIALS AND METHODS

Mice were obtained from the Roscoe B. Jackson Memorial Laboratory. They consisted of two strains: a black-agouti strain known as CBA/JAX (F77) which is homozygous for dominants at the C, B, and A loci, and an albino strain known as ST/JAX (F34) which is homozygous for the recessive alleles at these same loci. These mice and the lines derived from them, by brother-sister matings, were used in this experiment. At two months of age, males from the different strains were proven to be fertile by being paired with single females. In most cases the females were of their own strain, in two cases (pens No. 101, No. 104) both males were proven fertile by albino females, while in one case (pen No. 113) each male was proven

fertile by a female of the opposite strain. After the males were proven fertile, one of each strain was simultaneously placed in a cage that was new to both of them. The pens were clear plastic cages with dimensions 11½" by 7½" by 5" deep. Woodshavings were used as bedding material and the mice were given a diet consisting of Rockland Mouse Diet pellets, Ful-O-Pep Rabbit Ration Pellets and Delaware Scratch Grains. Food and water were available at all times.

After the males of the different strains had been together for 48-72 hours, an albino female of the ST/JAX strain was placed in the experimental pen. Thereafter the three mice remained together constantly. Litters were born in the cage, scored and then destroyed. Since those new born young which were fathered by black-agouti males have pigmented eyes while those fathered by albino males do not, the paternity of each offspring is apparent at birth. If a female died or showed signs of tumor formation, she was replaced. The replacement females were sometimes virgin and other times not. If, however, one of the competing males died, the remaining male was discarded and the cage was discontinued. Ten competition pens were established and each cage was scored for ten litters, giving a total of 100 litters. In order to speed up the obtaining of litters, in some of the later pens (pens No. 116, No. 118, No. 119, No. 122), more than one albino female was placed in the competition cage. In these instances the females, when pregnant, were removed to isolation cages. There, the litters were born and scored. After the litter had been recorded, the female was returned to the competition cage. The possibility that the various alterations of procedure, mentioned above, may have affected the results obtained will be discussed later. After the tenth litter had been obtained, the fertility of any male who had not fathered any young in either the 9th or 10th litter was checked. Only the results obtained from those competition pens that gave 10 litters and in which both males were shown to be fertile both at the beginning and at the end of the experiment are included.

RESULTS

The experimental procedure of placing an albino male and a black-agouti male in a cage with an albino female gave three types of litters. In one type of litter all the offspring were albino; in the second type all the offspring were pigmented; while the third type included both albino and pigmented offspring. This last type of litter is obviously the result of double inseminations. The data are shown in table 1 and figure 1. Table 1 presents the actual number of offspring, in each litter, that were fathered by the ST and CBA males; the total number of litters that were fathered solely by the albino males, the total number of litters fathered solely by the black-agouti males and the total number of litters fathered by both males (M = mixed litters). From these data the probabilities were computed for each pen, that the results, of the total number of litters fathered solely by the ST males as compared with the total number of litters fathered solely by the CBA males, could have been due to chance. For mixed litters, the table gives the num-

ber of offspring that were fathered by the ST males and the number of offspring fathered by the CBA males.

An examination of the table reveals several interesting facts. There are no significant differences in litter size in the three types of litters. The average sizes of the three types of litters were: 5.4 ± 1.6 for litters fathered solely by the ST males; 6.2 ± 1.7 for litters fathered solely by the CBA males; 5.4 ± 1.7 for those litters fathered by both males. While the number of mixed litters must obviously represent a minimum number, as some mixed litters may have gone undetected due to chance eating of young by the mother, it is noteworthy that the average size of mixed litters was not greater than that of the others.

The reproductive superiority of the ST (albino) males, under these experimental conditions, is quite obvious. In considering each experimental pen separately, one finds that the results of 6 of the pens have probabilities of chance occurrences of less than $p = .05$ (as tested by summation of binomial coefficients). One cage (pen No. 122) has a probability of chance occurrence quite close to the level of statistical significance. Although the results of 3 pens (pens No. 104, No. 105, No. 118) could easily have occurred by chance alone, it is interesting to note that in each case there were at least twice as many all-ST litters as all-CBA litters. The difference between the total of 76 all-ST litters and 12 all-CBA litters is highly significant ($p < .001$ by chi-square test). In considering the total number of offspring produced, one finds that out of a total of 552 mice born during the experiment, 458 were fathered by albino males while only 94 were fathered by black-agouti males. The difference here too is highly significant ($p < .001$ by chi-square test). Further evidence of the reproductive superiority of the ST males is shown by the results of the 12 mixed litters. In these litters there were more than twice as many offspring fathered by the ST males than by the CBA males. The difference between 46 ST offspring and 19 CBA offspring is also highly significant ($p = .0015$ by chi-square test). The reproductive superiority of the ST males is thus revealed by all three criteria. While the alterations of procedure mentioned earlier in this report may have produced some differences in the results obtained from the various cages, the consistency and one-sidedness of the data for all cages indicates that the effects of these variations in procedure must indeed have been small.

DISCUSSION

As mentioned earlier in this paper the phenomenon of sexual selection in mice has not been well studied. However, an experiment bearing on this problem, in rats, was reported by King (1929). In that experiment, King placed single, black male rats with either 1, 2, 3 or 4 albino male rats. These combinations of males were then placed in cages either with an albino or a black female rat. King found that mixed litters were some three times more frequent than pure (either all black or all albino) litters. She further found that there was a slight excess of offspring fathered by the males that were of opposite strain to that of the female used in the cage. She inter-

TABLE 1

RESULTS OF COMPETITIVE MATINGS BETWEEN MALES FROM DIFFERENT INBRED STRAINS OF MICE

Pen No.	Litter No. 1		2		3		4		5		6		7		8		9		10		Total No. of Litters				In Mixed Litters	
	ST	CBA	ST	CBA	ST	CBA	ST	CBA	ST	CBA	ST	CBA	ST	CBA	ST	CBA	ST	CBA	ST	CBA	ST	CBA	M	P	ST	CBA
101	8		6		8		7		7		3		2	2	5		5		6	1	8	0	2	.003	8	3
104	6		4		6			7	6	1	4			3	3		7		5	1	6	2	2	.144	11	2
105	6	2		10	2	2	7		6		4		3		5			6	3		6	2	2	.144	8	4
107	5		7		8		9		6		6		6		6		3		7		10	0	0	.001	..	..
113		7	6		4		6		4		4		5		5		5		6		9	1	0	.010	..	..
115		5	3		1	2	5		4		5		5		4		6		8		8	1	1	.019	1	2
116	3		3		6		6		7		4	1	3		7		6		3		9	0	1	.002	4	1
118	4		6			6		6	6		7		5		2	4		5	5		6	3	1	.253	2	4
119	7		8		6	1	6		4			7	7		2	1	4		5		7	1	2	.035	8	2
122		5	6		6		8		4		4	1	6			8	5		4		7	2	1	.089	4	1
Totals																					76	12	12		46	19

preted her results as indicating that in double mating experiments, with the rat, there is some form of discrimination in fertilization which strongly favors the union of gametes of unlike genetic constitution.

This is in sharp contrast to the findings of the present experiment in which the frequency of mixed litters is relatively small (12%) and where the pigmented males are definitely handicapped in reproduction. The mechanism by which this handicap of CBA males is achieved is unknown. It might be due to: (1) selective fertilization, which in this case would strongly favor the sperm of males of the same strain as that of the female, (2) a female preference for males of her own strain, (3) a male-male interaction in which

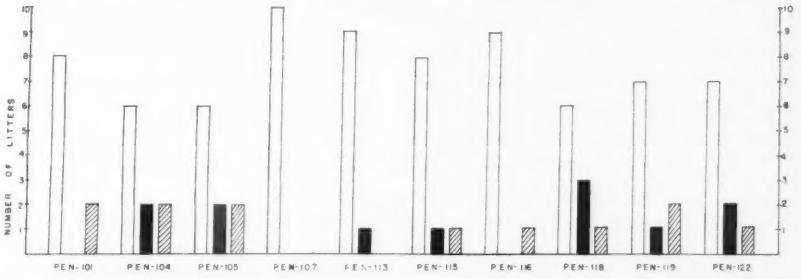

FIGURE 1. Results of competitive matings between males from different inbred strains of mice. White columns represent number of litters fathered solely by albino (ST) males. Black columns represent number of litters fathered solely by black-agouti (CBA) males. Cross-hatched columns represent number of litters fathered by both types of males (mixed litters).

social dominance, as achieved by fighting, leads to reproductive dominance (sexual selection). That social dominance leads to reproductive dominance has been shown for a number of animals (Alverdes, 1935; Carpenter, 1942). In a study of social hierarchy in mice, Uhrich (1938) found that within his strain of albino mice, the social hierarchy in some groups of mice was so rigid that only the dominant male was seen to mount the female. In the course of the present experiment, chance observations have been made of fighting between competing males. Observations were made of 27 fights which occurred within 6 of the cages. These observations were made over varying periods of time, up to six months. In 25 of these fights, the albino (ST) male was the aggressor while in only two of these fights was the black-agouti male the aggressor. Each fight was observed until the "submission reaction" (sitting on hindlegs, forelegs extended in defensive position) was exhibited by the vanquished male (Scott and Frederickson, 1951). It would appear that there is a correlation between fighting ability and reproductive efficiency.

The significance of any form of selection lies in its effects on the frequencies of genes in a population. Should the organism fail to reproduce because of some specific characteristic, structural or behavioral, that it may have, then the genes that produced this characteristic are lost to the population in the next generation. A correlation between social dominance and sexual selection simply means that the genes that govern one type of behavior, namely fighting, are also important in determining another type of

behavior, namely mating. This would then indicate that the breeding structure of mouse populations may be similar to the breeding structure of those organisms that show strong territoriality drives and rigid hierarchical systems. If true, this would be most remarkable as no territorial drive has been observed in wild house mice and the establishment of mating pairs, even for a given breeding season, has been observed in very few rodent species (Blair, 1953). Sexual selection may also help explain the persistence of certain genes (*t*) in wild mouse populations at frequencies that are in contradiction to the equilibrium frequencies postulated for them.

SUMMARY

Ten male mice of the albino strain, ST/JAX, were randomly paired with ten male mice of the black-agouti strain, CBA/JAX. Each pair was then placed in a cage with an albino female. Ten litters were obtained from each pen giving a total of 100 litters. All males were proven fertile both at the beginning and at the end of the experiment.

Three types of litters were produced: those containing only albino offspring, or only black-agouti offspring, or mixed (some offspring albino and some black-agouti). This last type of litter was the result of double inseminations. There was no statistical difference in litter size, among the three types of litters. It was found that 76 per cent of the litters were fathered solely by the albino males, 12 per cent of the litters were fathered solely by the black-agouti males, while 12 per cent of the litters were the result of double inseminations. Within the mixed litters, albino offspring were more than twice as frequent as black-agouti offspring. Of a total of 552 mice born in the ten cages, 458 were fathered by the albino males while only 94 were fathered by the black-agouti males. The evolutionary implications of sexual selection for mouse populations are discussed.

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MATING CALL IN THE SPECIATION OF ANURAN AMPHIBIANS*

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INTRODUCTION

Analysis of mating call has been used as a tool in the study of anuran speciation only in the past five years. It is somewhat premature to undertake a general synthesis of this work but progress has been made to the point where some generalizations and numerous problems for future study are readily apparent.

The mating call is an especially favorable characteristic of anurans for the investigation of speciational and general evolutionary dynamics in this group. This is true because, with few possible exceptions, the function of the call is limited to the attraction of a mate. The essentially voiceless females are attracted to the calls of the males which generally precede them to the breeding pools. It is to be expected, therefore, that evolution of the call would be influenced by two major factors: (1) the function of attracting a female of the same species; (2) the disadvantage population-wise of attracting a female of another species. It is to be expected that the first function would tend to promote uniformity of call through the species population and that the second would influence differentiation between species.

The foregoing statements about the function of the call are based on observational and circumstantial evidence. Experimental evidence for the specificity of call in attracting the females to the correct species of males has yet to be obtained largely because of the difficulty of setting up an experiment in the brief time in which ripe females are responsive to the males. Under the anuran system of assembling the sexes for reproduction, discrimination rests almost entirely with the female. A sexually excited male tends to clasp indiscriminately any object of appropriate size, sometimes even inanimate ones. Various sex recognition mechanisms have been developed which induce a male to release another male it has clasped (Noble, 1931). In toads (Bufo) a warning vibration serves this purpose (Aronson, 1944; A. P. Blair, 1947). There is no such mechanism to induce a male to release a female of the "wrong" species, and except where there is great disparity in size the determination of whether a mating will be homospecific or heterospecific in a mixed population depends on the discrimination of the female. I have seen female *Bufo valliceps* approach a calling male and hop in front of him. If the male fails to notice her she will

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repeat the performance until the male sees her and clasps. Lowe (1954) observed several instances of a female going to a calling male of her own species in a mixed chorus of two species of spadefoots (Scaphiopus). This same author found no heterospecific pairs among 200 clasped pairs in mixed populations of two species of Scaphiopus and two of Bufo observed on ten nights in New Mexico. Thornton (1955) found eight per cent of heterospecific pairs among 114 in mixed breeding populations of *Bufo woodhousei* and *B. valliceps*. Under the breeding system that pertains in the anura, occasional heterospecific combinations are to be expected as the result of a female passing too near the "wrong" male in responding to the call of a male of her own species. The probability of such mistakes occurring would be related to the concentrations, relative abundance, and dispersion of the pertinent species in the breeding pool.

While focusing our attention on mating call, we are aware that difference in call is only one of several factors that make up the isolation mechanism complex of any pair or group of sympatric species. Ecological factors such as time and place of breeding and mechanical ones such as relative size also may be involved. No one factor in the complex may be completely effective, while the combined effects of all factors may be wholly effective in preventing cross-mating.

The measurements of calls to be discussed here were made by use of a sound spectrograph (Sona-Graph). Use of this instrument provides a visual representation of sounds so that such variables as frequency, time, and modulation patterns may be measured precisely and compared as objectively as any morphological character. The acoustical terminology is made as consistent as possible with that of Chavasse, Busnel, Pasquinelly and Broughton (1954).

INTRASPECIFIC VARIATION

Variation within Populations. Some variations exist between the calls of individuals in a local population, and there are some differences between calls of the same individual. However, these variations fall within relatively narrow limits. Some of the factors responsible for these variations are known. One factor at least is non-genetic. W. F. Blair (1955 a) has shown that frequency increases with temperature in the calls of *Microhyla olivacea* and M. *carolinensis*. Our unpublished data indicate that this is generally true in other anuran calls. The repetition rate in those calls which consist of a series of repeated notes also tends to increase with temperature. Frequency tends to vary inversely with size in the anura. This is shown in Microhyla (W. F. Blair, 1955 a, 1955 b). Another microhylid, *Hypopachus cuneus*, with a structurally similar call is larger than either U. S. species of Microhyla and has a lower frequency than either. Repetition rate also tends to vary inversely with body size. This is true in toads of the United States (W. F. Blair, 1956 a) where the largest, *Bufo marinus*, has the slowest repetition rate and lowest frequency, and the others show increased rate and higher frequency as their size decreases. The dominant frequency ranges

from about 640 cps in *B. marinus* to about 5,000 cps in *B. quercicus,* which is the smallest toad. The repetition rate ranges from about 10 notes per second in *B. marinus* to about 120 notes per second in *B. debilis,* which is our smallest "trilling" toad.

The call may vary in relation to the sexual excitement of the individual. Various species of Hyla call from high in the trees in which they live. These tree calls are often given in daylight hours, and they may be heard at long distances from any breeding pool. We are able to compare the tree and breeding-pool calls of one species, *Hyla squirella,* by use of records obtained at Welaka, Putnam County, Florida. The breeding-pool call is about 0.2 seconds in length and consists of a series of closely spaced harmonics. The most distinctive feature of this call is a series of harmonically related emphasized bands which rise in frequency from beginning to end of the call and thus cut across the series of closely spaced harmonics (fig. 1). The tree call, as recorded for an individual about 20 feet high in a palm tree, is noise-like, without harmonic structure, and there is no rise

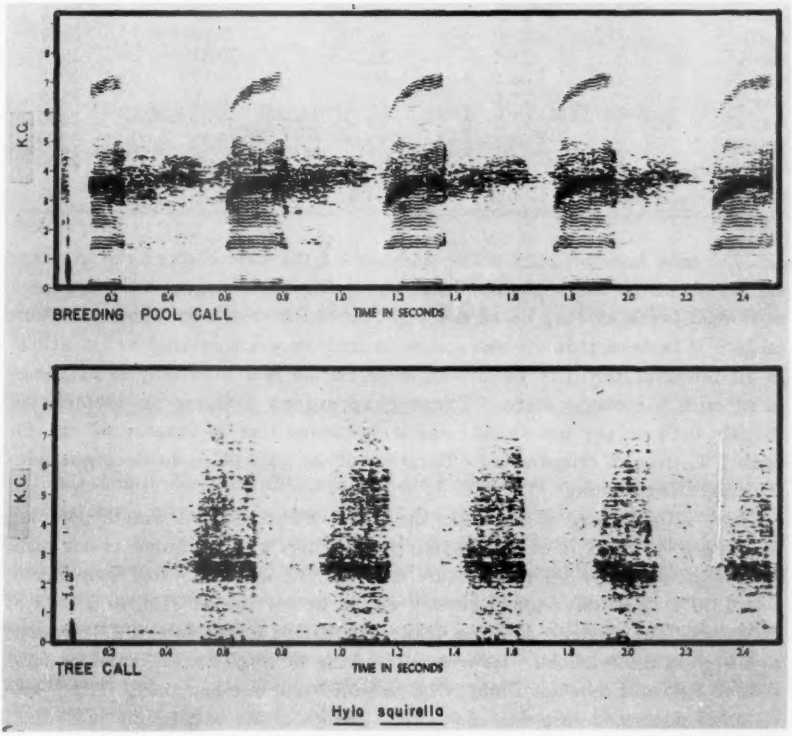

FIGURE 1. Sonagrams of breeding pool calls and tree calls of squirrel treefrog (*Hyla squirella*) recorded at Welaka, Putnam County, Florida. Note harmonic structure and rising frequency in breeding pool call.

in the dominant frequency band from beginning to end of the call (fig. 1). This call is of essentially the same length as the breeding-pool call. In comparison with the breeding-pool call, the tree call sounds flat and monotonous to the human ear. The function of the tree call is unknown.

The range of variation in calls of a single individual was investigated by recording the calls of a marked *Hyla versicolor* on nine different nights over a period of more than three months (table 1). In this time there was a range of 9°C. in ambient air temperatures at which the recordings were made. In this period the repetition rate varied by five notes per second from a low of 25 to a high of 30. The dominant frequency varied only 375 cps from a low

TABLE 1

MEASUREMENTS OF THE CALL OF A SINGLE INDIVIDUAL GRAY TREEFROG (*HYLA VERSICOLOR*) AS RECORDED ON 9 DIFFERENT NIGHTS.

Date	Air temperature °C	Mean pulses per second	Mean duration in seconds	Dominant frequency in cps
March 20	18.0	26.0	0.56	2250
April 2	23.0	28.0	0.69	2490
April 25	22.0	26.0	0.59	2500
May 15	24.0	25.5	0.51	2520
May 20	26.0	30.0	0.57	2625
May 21	27.0	30.0	0.30	2675
May 29	22.5	25.0	0.44	2600
July 6	26.0	30.0	0.38	2580
July 9	27.0	30.0	0.35	2600
Range of variation	9.0	5.0	0.39	375

of 2250 to a high of 2625. The duration of the call showed the greatest variation, for on the night on which the calls were longest they averaged more than twice as long as on the night on which they were shortest. From table 1 it is seen that the variations in frequency are related to variations in air temperature. The variations in repetition rate also tend to be correlated with air temperature. These observations indicate an increase of roughly 0.44 pulses per second and a frequency rise of roughly 60 cps for each 1°C. rise in temperature. Duration of the call tends to decrease with increased temperature.

There is individual variation in the calls of anurans in a single breeding population. The calls of 17 *Pseudacris streckeri* were recorded at one pond on a night when the air temperature was 21.5°C. and the water temperature was 18.0°. The dominant frequency varied from a low of 2100 to a high of 2500 cps. The average duration of the call varied from a low of 0.05 second to a high of 0.09 second. However, 11 of the 17 frogs had an average duration of 0.05 and four had 0.06. One had 0.07 and one had 0.09. There was virtually no measurable variation in the length of the call in any individual except the one which averaged 0.09 second. This frog had a range from 0.07 to 0.11 second. The average interval between calls varied from 0.40 to 0.45 second. The variation in interval in calls of a single individual was

slight. The difference between longest and shortest interval ranged from 0.02 to 0.14. The number of calls given in a sequence varied from six to 16 among the 17 individuals. Fifteen gave six, seven, or eight calls in a sequence. One gave 10 and another gave 16.

From the preceding discussions it is apparent that the anuran mating call is affected to a considerable extent by environmental factors, particularly temperature. In addition, there is some ontogenetic change in the call, since both frequency and repetition rate are related to body size. Differences other than those attributed to ontogenetic factors, environment or degree of sexual excitement are assumed to have a genetic basis.

Geographical Variation. Minor differences in call exist between local subpopulations, and for a few species there is sufficient evidence to show broader patterns of geographic variations of this character. *Microbyla carolinensis* and *M. olivacea,* which overlap along the forest-grassland boundary in Texas and Oklahoma, show geographical variations in dominant frequency and duration of the call that are such that the greatest differences in calls of the two species occur in their overlap zone (W. F. Blair, 1955a). The variation in frequency is correlated with variation in body size (W. F. Blair, 1955b).

TABLE 2

GEOGRAPHIC VARIATION IN THE MATING CALL OF THE CHORUS FROG (*PSEUDACRIS NIGRITA*).

Attributes	Texas	Oklahoma	Florida	Colorado	Minnesota-Wisconsin
Pulses per second	18.8	20.9	28.6	31.2	28.6
Dominant frequency (cps)	2969	3075	3325	2275	3820
Duration in seconds	0.68	0.58	0.39	0.43	0.49
Number of individuals	13	2	3	2	10

Limited data indicate that the call of *Pseudacris nigrita* is different in the northern United States from that of the same species in the South. The calls of 13 individuals from five stations in southeastern Texas have an average repetition rate of 18.8 pulses per second (table 2). The calls of two individuals from northeastern Oklahoma are closely comparable to these, with an average of 20.9 pulses per second. The calls of 10 individuals from northwestern Minnesota and northwestern Wisconsin have a higher repetition rate, with an average of 28.6 pulses per second. Two individuals recorded at an elevation of above 9000 feet in Rocky Mountain National Park, Colorado, have an average repetition rate of 31.2 pulses per second, which is comparable to the rate in the northern populations. When the probable effects of temperature are considered, the differences between the

northern, including Rocky Mountain, and Texas-Oklahoma frogs are presumably greater than the raw data indicate. The northern frogs were recorded at temperatures of 18.0 to 19.5°C, while the Texas-Oklahoma frogs were recorded at temperatures ranging from 18.5 to 24.0°C. The temperature effects would thus tend to obscure genetic differences in repetition rate between these geographic segments of the species population. Three individuals recorded on the mainland near Cedar Key, Florida, at an air temperature of 26.5°C., have an average repetition rate of 28.6 pulses per second, which suggests that the peninsular Florida population differs in call from

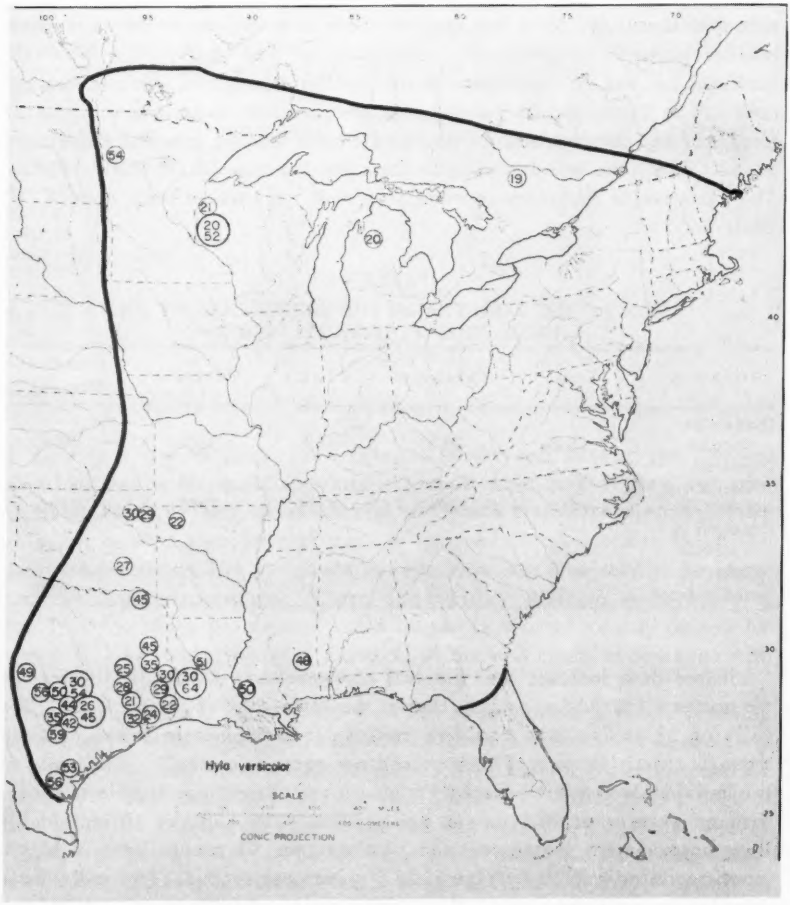

FIGURE 2. Geographic variation in pulse rate in the call of the gray treefrog (*Hyla versicolor*). Figures given are station means for pulses per second. Circles enclosing two values represent stations at which discrete "fast-trilling" and "slow-trilling" types were recorded.

the Texas-Oklahoma segment. The Texas-Oklahoma and Florida frogs show little difference in dominant frequency, but their frequency is decidedly lower than that of the Minnesota-Wisconsin frogs (table 2). The lowest frequency is found in the Colorado frogs. The duration of the call is longest in the Texas-Oklahoma frogs, shortest in the Colorado and Florida frogs, and intermediate in the Minnesota-Wisconsin ones. The three groups, Colorado-Minnesota-Wisconsin, Texas-Oklahoma, and Florida, represent three subspecies which have been described on morphological grounds. Much additional work is needed, however, before it will be possible to determine the degree of correlation between geographically varying call characters and geographically varying morphological characters in this widely distributed species.

TABLE 3

MEAN MEASUREMENTS OF THE CALLS OF THE GRAY TREEFROG (*HYLA VERSICOLOR*). STATION SAMPLES POOLED TO FORM A SOUTHERN TRANSECT FROM CENTRAL TEXAS TO EASTERN MISSISSIPPI AND A NORTHERN TRANSECT FROM NORTHWESTERN MINNESOTA TO EASTERN ONTARIO.

Atributes	Southern transect			Northern transect	
	Southwestern fast trillers	Southern slow trillers	Southeastern fast trillers	Northwestern fast trillers	Northern slow trillers
Pulses per second	47.3	26.8	50.8	52.4	20.3
Dominant frequency (cps)	2683	2435	2643	2650	2407
Duration in seconds	0.59	0.60	0.77	0.56	0.60
Number of individuals	28	33	20	6	9

The most complete analysis of geographic variation in call can be made for the gray treefrog, *Hyla versicolor,* although much remains to be done before the complete pattern becomes clear. The evidence consists of a southern transect across a part of the range from central Texas to eastern Mississippi and a northern transect from northwestern Minnesota to eastern Ontario (fig. 2). Repetition rate shows an extremely interesting pattern of distribution. Frogs at the extreme western periphery of the range have a rapidly trilled call. In the south these western frogs have an average repetition rate of 47.3 pulses per second (table 3). Frogs in western Louisiana and in the pine-oak forest of eastern Texas, and in the Ouachita Mountains of eastern Oklahoma and Ozarks of eastern Oklahoma and western Arkansas have a slowly trilled call with an average repetition rate of 26.8 pulses per second. Frogs from western Louisiana east to eastern Mississippi have a rapidly trilled call with an average repetition rate of 50.9 pulses per second. Thus there are eastern and western rapid-trilling populations separated by

a slow-trilling population in western Louisiana and eastern Texas. Points of contact between both fast-trilling populations and the intervening slow-trilling segment have been determined. At the known points of contact (fig. 2), slow-trilling and fast-trilling frogs occur in the same breeding aggregation without any evidence of intermediacy of call. The sharp segregation into two call types suggests that a single gene difference might be responsible for the two types. In Texas the contact occurs in the belt of transition from pine-oak forest to grassland. One known point of contact between the western and central populations is about two miles northwest of Columbus, Colorado County, Texas, and another is 6.5 miles southeast of Bastrop, Bastrop County, Texas. In northern Texas, where the forest-grassland boundary swings northeastward, both types were found calling together about 7.7 miles north of Clarksville, Red River County, but only the fast triller could be recorded. Recordings were made of slow-trilling frogs (35 pulses per second) 10 miles southwest of Nacogdoches, Texas, on June 30, 1953. Recordings of fast-trilling frogs (45 pulses per second) were made on May 5, 1956, five miles southwest of Nacogdoches. The difference between these two groups is somewhat minimized by the 7°C. difference in the temperatures at which they were recorded. When the calls are corrected to 22.0°C. by adding or subtracting 0.44 notes per second for each 1.0°C. change in temperature the relationship is 45.8 pulses per second for fast trillers and 32.0 for the slow trillers. No records are available for the western part of the range of the species in Oklahoma to show whether or not there is a fast-trilling population there.

The one known contact between the eastern, fast-trilling population and the slow-trilling one is near Caney, Vernon Parish, Louisiana. Two individuals in a stock collected there by Floyd E. Potter, Jr., later called in a natural situation at Austin. One of these had a repetition rate of 30.0 pulses per second at 27.0°C., and the other had a rate of 64.0 pulses per second at 29.0°C.

The northern transect is similar to the southern one except that no eastern, fast-trilling population is known in the area from which recordings are available. The only known contact between the fast and slow-trilling populations in the north is near Clam Lake, Burnett County, Wisconsin. Only slow-trilling frogs were found in a breeding pool near Clam Lake, but both types were found in a rainpool in a cleared field in an upland situation about two miles away (fig. 2).

There is a wide gap in our knowledge of the distribution of call types in the central United States. The occurrence of the fast type in Minnesota and Wisconsin as well as in Texas suggests that it is distributed along the western periphery of the range of the species. Charles F. Walker has told me of hearing two types of calls in Ohio, which is not unexpected since the forest-grassland border swings far eastward in that latitude.

The fast-trilling populations average about 200 cps higher in their dominant frequency than do the slow trilling ones (table 3). There is little

geographic variation in the duration of the call. The longest calls are found in the southeastern, fast-trilling populations.

No one of the several possible hypotheses that might explain the geographical call races of *Hyla versicolor* can be proved on the basis of present evidence. One possible explanation is that *Hyla versicolor* as now recognized actually includes three species of which all meet and overlap the adjacent species without interbreeding. This needs to be tested by breeding experiments and call discrimination experiments, but it seems the most unlikely of several hypotheses. We have been unable to see any morphological differences between individuals of the two call types where they occur together, nor is there any good evidence of ecological separation of the two types in time or place of breeding where they overlap. Another possible explanation would be that the slow-trilling characteristic arose in the center of the range and spread from there. However, it would be difficult to envision any advantage of this call that might favor its spread, and it would seem most likely that a change in call would be at a selective disadvantage and would thus have its spread inhibited. An alternative hypothesis would be that the fast-trilling type arose at the periphery of the range and became established under the conditions of limited gene flow at the periphery. This hypothesis would require that the character arose at least twice, once on the western and once on the eastern side of the range, and it would require numerous origins of the trait at the periphery or a directional dispersal of the character along the periphery.

The most likely hypothesis is that the contacts between the slow-trilling population and the fast-trilling populations to the east and west of it are secondary. These contacts in the South occur in a region in which there is widespread evidence of previous (presumably late Pleistocene) fragmentation of ranges into southeastern and southwestern segments which still remain separate, have evolved into eastern and western species which now overlap in range, or show secondary interbreeding (W. F. Blair, 1951). Pleistocene separation of the ancestral population into southeastern and southwestern fragments which remained undifferentiated in call and concomitant isolation of an intervening population which chanced to undergo differentiation of the call under geographic isolation would be required. Subsequent rejoining of the ranges of the three populations and their northward spread prior to the evolution of effective isolation mechanisms would lead to the situation that exists today. The postulate of a Pleistocene isolate on the western Gulf Coast is not without supporting evidence. A relict toad, *Bufo houstonensis,* is limited to the southeastern Texas coastal area and is disjunct by the distance from there to the Pearl River, Mississippi, from the range of its near relative *Bufo terrestris.* The three populations of gopher frogs, usually referred to as *Rana areolata, R. sevosa* and *R. capito,* although Schmidt (1953) has lumped the latter two under a single species name, demonstrate the occurrence of three Pleistocene isolates on the Gulf Coast comparable to what has been hypothesized above as an initial step toward the present situation in *Hyla versicolor.*

The geographical distribution of the call races of *Hyla versicolor* does not show any general concurrence with the distribution of the three rather nebulously defined morphological subspecies which have been described for this species. The only exception is that the contact between the western, fast-trilling race in the south with the central, slow-calling race occurs at about the eastern boundary of the subspecies *H. versicolor sandersi,* Smith and Brown (1947).

The limited evidence that we now have pertinent to geographic variation in mating call suggests that with adequate geographic sampling this character will prove an extremely valuable tool for revealing past history and evolutionary dynamics of complex, widely distributed species. The main value of this character in this respect lies in the fact that variations in call, with some exceptions, are largely independent of environmental selection. Natural selection could affect call secondarily, however, through its effects on body size, since certain attributes of the call do vary in relation to size. Also, as will be discussed below, call could be influenced by selection under conditions of limited hybridization between populations which had evolved a set of partially effective isolation mechanisms.

INTERSPECIFIC VARIATION

Call analyses are now available for all but a few species of U. S. anurans with the exception of the genus Rana. With minor exceptions which will be discussed below, each species differs distinctly from every other species in at least one, and often more, of the attributes of the call. Nevertheless, groups of species within a genus show basic similarities in call structure, which, along with other evidence, are indicative of evolutionary relationship.

Analysis of call in the genus Scaphiopus (W. F. Blair, 1955 c, 1956 b) supports the morphological evidence and the evidence from breeding tests (A. P. Blair, 1947 b; Wasserman, 1957) that there are two distinct evolutionary lines (subgenera) in the genus. Comparison of the calls in the subgenus Spea leads to the suggestion that there are three U. S. species (W. F. Blair, *op. cit.*). The species *hammondi* and *bombifrons,* which are differentiated in repetition rate, have overlapping ranges and show limited hybridization where they occur together. The species *intermontanus* appears to be an allopatric species most closely related to *bombifrons.*

A preliminary grouping of U. S. toads (Bufo) into species groups (W. F. Blair, 1956 a) is already in need of revision in the light of additional work with call and with breeding tests. On present evidence, three species do not seem closely related to any other U. S. species and will be treated as representing three species groups. These are *B. valliceps, B. marinus,* and *B. quercicus.* One group includes two species, *B. compactilis* and *B. cognatus.* The largest group includes five nominal species, *B. terrestris, B. americanus, B. hemiophrys, B. houstonensis,* and *B. microscaphus.* The ranges of the first two meet, and they are generally treated as conspecific.

The other three are disjunct allopatric populations to the west of the range of these two and apparently represent relicts from a wider, Pleistocene distribution of the group (A. P. Blair, 1955; W. F. Blair, 1957a, 1957b) Another species, *B. punctatus,* appears closely related to this group and presumably speciated prior to the evolution of the allopatric species mentioned above. Another species, *B. woodhousei,* which occurs sympatrically with all of the species of this group except *B. hemiophrys,* has the call markedly differentiated (W. F. Blair, 1956a), but it is genetically so similar that hybrids between it and members with which it has been tested are fertile (A. P. Blair, 1941). Another species, *B. debilis,* has a call that is intermediate in structure between *B. woodhousei* and *B. americanus.* Viable metamorphosed hybrids between *B. debilis* and *B. terrestris* have been produced in my laboratory.

The final species group includes *B. boreas, B. canorus,* and *B. alvarius,* although the evidence for placing the latter species in this group is rather slight at the present time.

Analyses of call in U. S. hylids are now being made along with breeding tests to determine the relationships of the species in this group of frogs. One clear-cut group includes *Hyla cinerea, H. gratiosa,* and *H. andersoni.* Another group includes *H. femoralis* of the southeastern United States and *H. arenicolor* of the Southwest. Another includes *Hyla versicolor* and *H. phaeocrypta* with close affinities to the *femoralis-arenicolor* group.

Detailed studies of call characteristics and variations within and between members of a species group should provide evidence as to how the call differences originated and consequently throw light on the mode of origin of the species. The evidence will be examined as it applies to two currently held theories as to how new species originate. One of these, favored by Muller (1940), Mayr (1943) and others, holds that a new species originates under geographical splitting of a population and continued separation until the two populations have differentiated to the extent that they would behave as separate breeding systems if their ranges again came into contact. Another theory (Dobzhansky, 1940) holds that incipient isolation mechanisms may be reinforced through the action of natural selection under limited hybridization of the two populations.

Allopatric Species. The populations known as *Pseudacris streckeri* and *P. ornata* provide one of a few U. S. anuran examples of a presumably early stage of geographic speciation. The former occurs west of the forest boundary in Texas and Oklahoma except for a relict population in southern Illinois. The latter species occurs throughout Florida and on the coastal plain from North Carolina to southern Mississippi. The few calls of *P. ornata* available for analysis show no detectable difference from the calls of Texas *P. streckeri* other than a 400 cps difference in dominant frequency. Both nominal species breed in winter and both are morphologically rather similar. Mecham (1957) obtained a high percentage of metamorphosis of viable hybrids in crosses between them. One attempted backcross of an

apparently mature male to a female *P. streckeri* gave inconclusive evidence of hybrid sterility. These two populations are apparently in an early stage of speciation, and it is doubtful from present evidence that they would behave as separate species if their ranges came to overlap in nature.

Two spadefoots, *Scaphiopus hurteri* and *S. holbrooki,* have a distribution very similar to that of the two Pseudacris. No calls of the latter species are available for comparison, but both nominal species sound very similar as heard by the human ear. Wasserman (1956) found these two to be interfertile populations which are apparently separated by a soil barrier in the Mississippi Valley. The stage of speciation of these two appears to be similar to that of the two Pseudacris, and it seems likely that the same past climatic change was responsible for the fragmentation of ranges in both groups.

The species *Hyla arenicolor,* with a range from Trans-Pecos Texas westward, and *H. femoralis,* with a range from the Mississippi River eastward on the coastal plain, present a different picture. These two species are adapted to life in very different environments. Body size is considerably greater in *H. arenicolor* than in *H. femoralis.* Hybrids between the two have survived through metamorphosis, but they were lost through faulty technique before they could be tested for fertility. The calls of these two species are more differentiated than those of the species pairs discussed above. The calls of both are rather long, ranging from about one to more than three seconds in length. In each species the call consists of a series of notes repeated at an approximately similar rate (15 notes per second in *H. arenicolor,* 12 notes per second in *H. femoralis).* Each note is made up of a series of harmonics of the fundamental, which is about 100 cps in *H. femoralis* and about 175 cps in *H. arenicolor* (fig. 3). The chief difference is in the bands of emphasized harmonics. In *H. femoralis,* there is an emphasized band at about 200 cps and harmonically related bands centering around 2600 and 4800 cps. The third (4800 cps) of these bands is usually the strongest, but the second sometimes exceeds it. In *H. arenicolor,* there is an emphasized band centering around 600 cps and another, usually stronger, band centering around 2400 cps. The call differences between these allopatric species are at least as great as those between the sympatric species *Hyla cinerea* and *H. gratiosa.*

The five allopatric species of the *Bufo americanus* group provide a final series of examples of call differentiation in geographically separate species. The calls of *B. terrestris, B. americanus,* and *B. houstonensis* have been described by W. F. Blair (1956a), those of *B. hemiophrys* and *B. americanus* by W. F. Blair (1957a), and those of *B. microscaphus* by W. F. Blair (1957b). Considering *B. terrestris* and *B. americanus* first, both species show geographical variation in call (fig. 4). At the extremes of this variation, *B. terrestris* of northern Florida has an average repetition rate of 74 notes per second and a duration of 4.8 seconds by comparison with a repetition rate of only 30 notes per second and a duration of 10.6 seconds for *B. americanus* in northeastern Oklahoma and northwestern Arkansas. A

single individual available from the western extreme of the range of *B. terrestris* has a longer call and slower repetition rate than does the species in Florida. In the northern part of its range, *B. americanus* has a faster repetition rate and shorter call than in Oklahoma and Kansas.

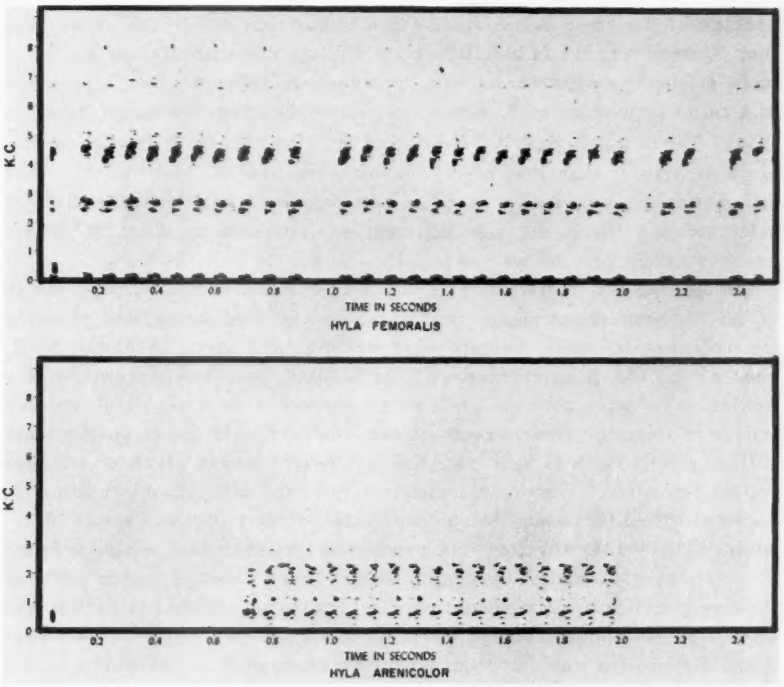

FIGURE 3. Sonagrams of the mating call of *Hyla femoralis* from Perry, Taylor County, Florida, and *H. arenicolor* from Baker Reservoir, Washington County, Utah. Note higher dominant frequency in the former.

In the southern disjunct population, *B. houstonensis,* the call is more similar to that of Oklahoma-Arkansas *B. americanus* than to Mississippi *B. terrestris,* which suggests that this allopatric species represents a relict from a former (possibly late Pleistocene) distribution of *B. americanus* southward west of the Mississippi Embayment.

The call of *B. hemiophrys* is strongly differentiated from the call of *B. americanus* in repetition rate and in duration (fig. 4). The ranges of these species essentially meet or possibly overlap in western Minnesota (W. F. Blair, 1957 a). The greater differentiation in call between *B. hemiophrys* and *B. americanus* than between *B. houstonensis* and *B. americanus* possibly indicates an earlier splitting off of the former than the latter population.

The southwestern relict species, *B. microscaphus,* has a short call comparable in duration to the call of *B. hemiophrys* (fig. 4) but with a repetition rate comparable to that of northern *B. americanus.* The dominant frequency is lowest (averaging 1300 cps) in *B. microscaphus* and next lowest (averaging 1548 cps) in *B. hemiophrys.* This compares with frequencies averaging from 1706 to 2300 cps in the samples available from the three remaining species of the group. The similarities in duration and frequency suggest that *B. microscaphus* is a relict from a Pleistocene extension of the range of *B. hemiophrys* southward. This hypothesis is supported by the presence of a relict population of *B. hemiophrys* in southeastern Wyoming (Stebbins, 1954). The morphological differentiation of *B. microscaphus* from *B. hemiophrys* is greater than that of *B. houstonensis* from *B. americanus.* This differentiation, principally in color pattern, could have proceeded under selection for life in the very different environments in which *B. microscaphus* and *B. hemiophrys* now exist.

Summing up the evolutionary relationships of the allopatric populations of the *B. americanus* group, the oldest speciational event was probably the splitting off of a western short-calling population ancestral to *B. hemiophrys* and *B. microscaphus.* At a later time, a withdrawal of the western population northward left *B. microscaphus* as a series of isolated relicts in tolerable environments in the Southwest. In the East, the long-calling population was split into the populations known as *B. americanus* and *B. terrestris.* The present distribution of the latter is of the kind associated with a presumed Pleistocene glacial-stage refuge in Florida (W. F. Blair, 1951). This splitting was possibly on an east-west basis, with the *B. americanus* population retreating to the Texas coast or farther south at the time the *B. terrestris* population was presumably isolated in Florida and later spreading northward and eastward to its present range. This theory of the distribution would account for the presence of *B. houstonensis* on the Texas coast as a relictual population most closely similar to Oklahoma-Arkansas *B. americanus.* The ranges of the *B. americanus* and *B. terrestris* populations came into contact (fig. 4) in the course of their spread, but the relationships of the two where this has occurred are still in need of clarification.

The splitting off of other groups such as the *boreas-canorus-alvarius* complex, *B. woodhousei, B. debilis,* and *B. punctatus* presumably occurred at a considerably earlier time than the speciational events discussed above.

Summarizing what we know about differentiation in call among allopatric species of anura, there is variation in degree of differentiation. The degree of differentiation is presumably related to the length of time that the isolation has existed. Some allopatric species differ just as much in call as do some sympatric species.

Sympatric Species. The sympatric species pairs available for analysis fall into two groups. In one group the distribution of call characteristics in the respective populations is such and the relations of the two popula-

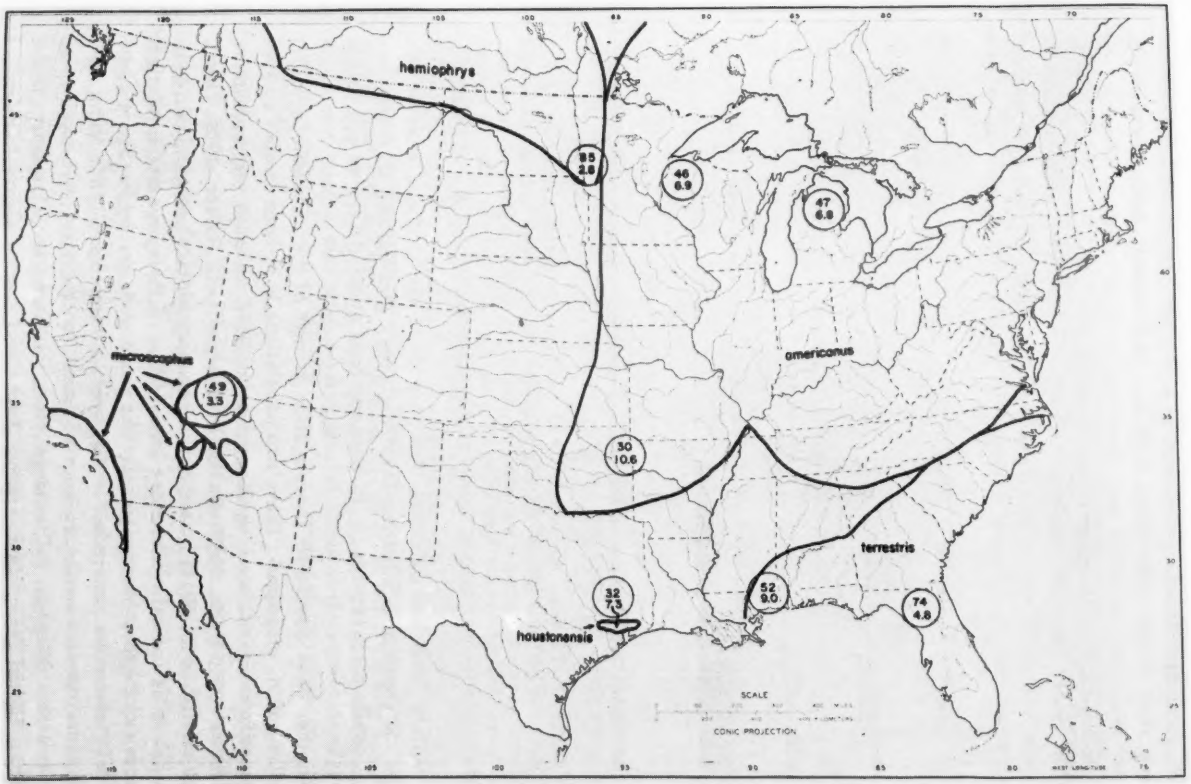

FIGURE 4. Variation in pulse rate and duration of the mating call in the *Bufo americanus* group of toads. Values given are station means. Top value shows pulses per second; bottom shows duration in seconds.

tions in the overlap zone are such as to suggest that reinforcement of partial isolation mechanism complexes has occurred and is occurring. In the other group the situation in respect to call characters is such that it suggests such strong differentiation of call and other isolation mechanisms under geographic isolation that there was no opportunity or necessity for reinforcement after the ranges became sympatric.

The most thoroughly analyzed situation in the first group is that of *Microhyla olivacea* and *M. carolinensis* (W. F. Blair, 1955a). The calls of the two species differ more in frequency and duration in the overlap zone

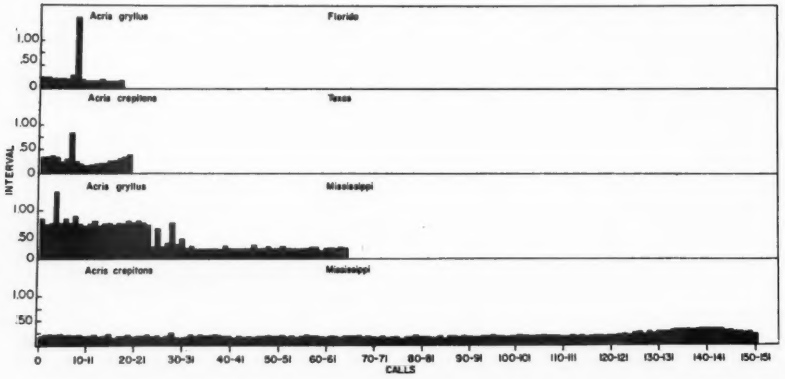

FIGURE 5. Pictorial representation of the intervals between calls in the call sequences of the cricketfrogs, *Acris gryllus* and *A. crepitans*. Intervals expressed in seconds between consecutive calls. Diagrams are for representative individuals. Mississippi individuals are from same breeding pool at Westonia, Hancock County. Note differentiation in respect to interval where the two species occur together.

in eastern Texas and Oklahoma than they do to the east and west of this zone. A comparable situation involving *Crinia glauerti* and *C. insignifera* in Australia discovered by A. R. Main has been quoted by Brown and Wilson (1956).

Another case now under investigation is that of the cricketfrogs, *Acris gryllus* and *A. crepitans*. These two populations have been treated as subspecies in recent taxonomic works (Schmidt, 1953), although various authors (Viosca, 1923; Dunn, 1938; Bushnell, Bushnell and Parker, 1939; Orton, 1947; Neill, 1950, 1954) have pointed out morphological, ecological, cytological or mating call differences as evidence of their behavior as distinct species where they occur together. The range of *A. gryllus* is that of a presumed Pleistocene isolate in Florida, being restricted to Florida and a strip along the coast from the vicinity of Lake Pontchartrain in Louisiana to southeastern Virginia. The range of *A. crepitans* includes most of the eastern United States from western Texas and eastern Colorado eastward except for the northeastern states and most of the range of *A. gryllus*. The two populations have been reported to occur together without interbreeding

in western South Carolina, Georgia, Alabama, northern Florida, Mississippi and Louisiana (Neill, 1954). The reports of Neill (1950, 1954) indicate an overlap area in Georgia from about 60 miles north of the Fall Line to northern Oklaloosa County, Florida.

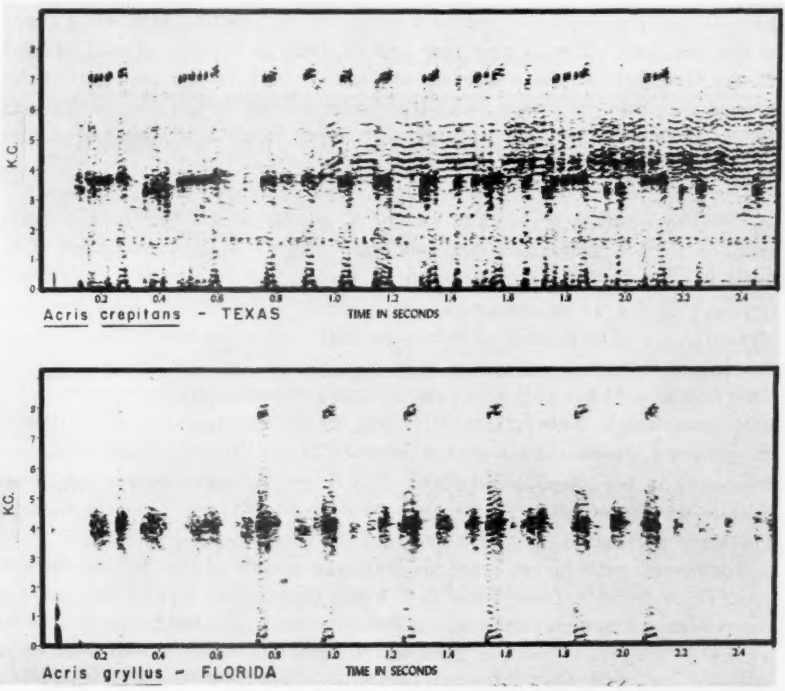

FIGURE 6. Sonagrams of calls of *Acris crepitans* from Austin, Texas, and *A. gryllus* from Welaka, Florida. The individual calls of the former are seen against a background of calls of other species. Note pulsed structure of first three calls. Calls of *A. gryllus* seen against background of others of the same species. Note similarity between the two species except for absence of pulsations in calls of *A. gryllus*.

Our present material consists of recordings in a mixed population in southwestern Mississippi (ghost town of Westonia, Hancock County), a series of recordings of *A. crepitans* from Texas, Oklahoma and Louisiana, and a few recordings of *A. gryllus* from peninsular Florida. The calls of Florida *A. gryllus* and western *A. crepitans* show only slight differences (figs. 5, 6). Where the two species occur together in southwestern Mississippi, however, their calls differ in every measurable characteristic. One way in which the calls differ is in the pattern of time intervals between calls in a call sequence. In the terminology we are using for the calls of these frogs, each individual note is regarded as a call; a series of calls is referred to as a call sequence. In the mixed population, *A. crepitans* has

a rapidly repeated series of calls, with very little variation in interval between them (fig. 5). In the call sequence of *A. gryllus* in the mixed population, there is a slow repetition of calls in the initial part of the sequence and a rapid repetition in the closing segment. The individual *A. gryllus* figured had an average interval of 0.65 second separating the calls in the first half of the sequence and an average of 0.22 second separating those in the last half. The *A. crepitans* had an average interval of 0.21 second in the first half of the sequence and one of 0.21 in the last half of the sequence. This difference in call between the two species in the overlap zone was first called to my attention by Percy Viosca, Jr., (oral communication).

The calls of the two species in the mixed population also show a striking qualitative difference. The call of *A. gryllus* is a simple note comparable to that of western *A. crepitans* and Florida *A. gryllus.* That of *A. crepitans* in the mixed population is broken into a series of elements (pulses) in the same manner as the "trilled" calls of various species of *Hyla* (fig. 7). The number of pulses per call increases from two or three in the initial part of the sequence to as many as 10 in the closing part of it. This breaking of the call into pulses occurs occasionally in western *Acris crepitans,* which, however, usually have a call structure similar to that of *A. gryllus* (fig. 6). There also appears to be a difference in the dominant frequency in the mixed population. Ten *A. gryllus* have an average dominant frequency of 3480 cps (range 3400 to 3600). Three *A. crepitans* from the mixed population have an average of 4083 cps (range 4000 to 4200).

Additional work is projected to learn the nature of the call of the two species of Acris in other areas inside and outside the area of overlap, but the present sample indicates strong differentiation. On the basis of present evidence, therefore, the two species of Acris are like the two species of Microhyla (W. F. Blair, 1955 a) in showing strong differentiation of the mating call where their ranges overlap.

The mixed population in southwestern Mississippi was found in a pool formed by the outflow from an artesian well. Aggregations of *A. gryllus* were heard in roadside ditches and other situations in this area, but *A. crepitans* was found only in and near the artesian water, which is a distributional situation previously called to my attention by Percy Viosca, Jr., (oral communication). One of the morphological characters mentioned by Dunn (1938) to separate the two species, the length and degree of webbing of fourth toe on the hind foot, served completely to separate the two species from the mixed population independently of call characters. In other words, all of the individuals having the *A. gryllus* type of call had long, less-webbed toes, and those with the *A. crepitans* call had short, more fully-webbed toes.

The three situations discussed above are readily explainable only by the theory that selection against hybridization in the overlap zone has served

to sharpen call differences and thus increase the effectiveness of this isolation mechanism. The only alternative explanation for the increased differences in call in the overlap zone would be that the differences are purely fortuitous, but this explanation seems hardly defensible.

The group of sympatric species in which there is no evidence of reinforcement of call differences includes such pairs as *Bufo compactilis-B. cognatus, Hyla cinerea-H. gratiosa,* and *Hyla versicolor-H. phaeocrypta.*

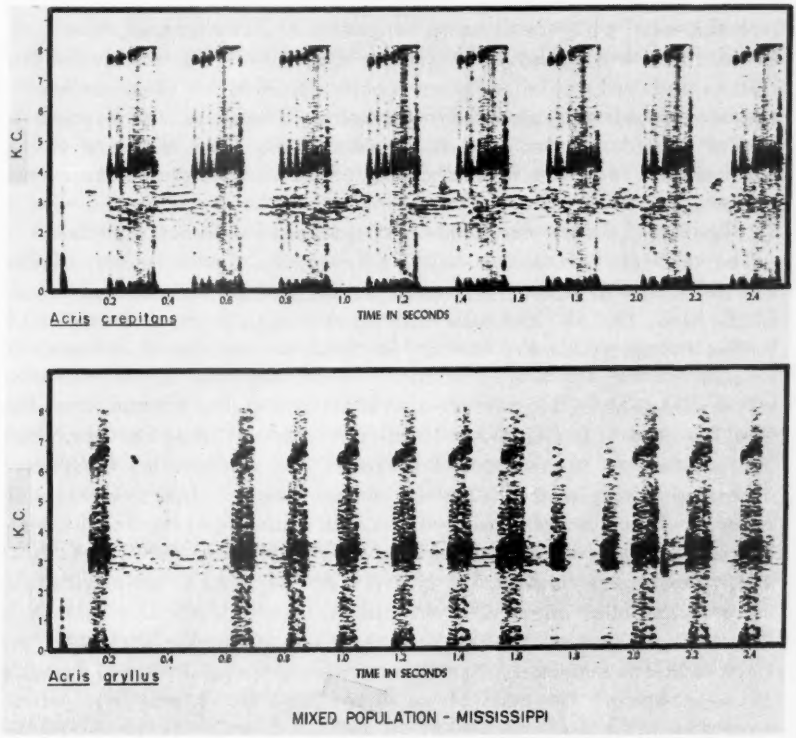

FIGURE 7. Sonagrams of calls of two species of Acris from same breeding pool at Westonia, Hancock County, Mississippi. Note difference between pulsed call of *A. crepitans* and unpulsed of *A. gryllus*. Calls of both seen against faint background of other species.

The ranges of *Bufo cognatus* and *B. compactilis* overlap from southwestern Kansas south through western Oklahoma, western Texas, and eastern New Mexico into Mexico. The calls of these two species are strongly differentiated in duration and to a lesser degree in repetition rate. The call of *B. compactilis* averages about one-half second in duration, and no call has been recorded in excess of 1.7 second. This short call is incessantly repeated. The call of *B. cognatus* is long, averaging about 20 seconds and

ranging up to 54 seconds in length. We can find no significant differences between calls of *B. compactilis* from the overlap zone in trans-Pecos Texas and those of the same species in the Austin region, some 250 miles east of the overlap zone. Nor are there any significant differences in call between *B. cognatus* from the overlap zone in the Texas panhandle and individuals from western Minnesota, some 500 miles northeast of the overlap zone.

There is no indication of reinforcement of call differences between *Hyla cinerea* and *H. gratiosa* where they occur together. Both species have typical coastal plain distributions suggestive of Pleistocene speciation in Florida, and their initial separation could have occurred on different glacial-stage islands in what is now peninsular Florida. The species *H. gratiosa* extends westward to southeastern Louisiana. The species *H. cinerea* extends westward to south-central Texas and northward in the Mississippi Embayment to southern Illinois. The latter species occurs throughout the range of *H. gratiosa* and extends beyond it on the west, in the Mississippi Embayment, on the northeast, and in southern Florida.

The calls of *Hyla cinerea* and *H. gratiosa* are structurally very similar and differ chiefly in the distribution of energy in the frequency spectrum (W. F. Blair, 1957c). The calls of these species differ from those of other U. S. treefrogs except *H. andersoni* in that a large number of harmonics of the fundamental are more or less equally emphasized. The fundamental (about 450 cps) in *H. gratiosa* is noticeably stronger, however, than the other harmonics. In *H. cinerea,* the eleventh and twelfth are usually somewhat emphasized in relation to the others. The result of this distribution of energy is to give the call of *H. cinerea* a higher pitch than that of *H. gratiosa.* Five calls of *H. cinerea* from four stations in central and eastern Texas have an average dominant frequency of 2860 cps (2700 to 3000) by comparison with a frequency of 2973 (2850 to 3150) in 11 individuals from southwestern Mississippi and 3470 cps (3200 to 3850) in five individuals from northern Florida. The slightly lower average frequency in the Texas frogs cannot be considered significant in view of the small size and overlap of the samples. The calls of the Texas frogs are shorter (0.11 second) than those of the Mississippi (0.17 second) and Florida (0.16 second) frogs. However, the length of the call in the Mississippi and Florida frogs is more like the length of the call of three *H. gratiosa* (0.16 second) from eastern Mississippi than is the call of the Texas frogs from outside of the overlap zone.

The range of *Hyla phaeocrypta* (= *avivoca*), which extends on the Gulf coastal plain from South Carolina to the Mississippi River in Louisiana, falls entirely in the range of *H. versicolor.* The two species are morphologically quite similar, although *H. versicolor* is somewhat larger than *H. phaeocrypta.* As in *Bufo compactilis* and *B. cognatus,* the calls of these two species of treefrogs are strongly differentiated. The calls of 10 *H. phaeocrypta* recorded in Perry County, Mississippi, average 1.99 seconds in length and have an average repetition rate of only 9.7 notes per second.

The call race of *H. versicolor* from the same region (table 3) has an average call length of only 0.77 second and a repetition rate of 50.8 notes per second. In addition, *H. phaeocrypta* has evolved a complex modulation pattern (W. F. Blair, 1957c) that is quite different from the simple pattern of *H. versicolor.* While the call race of *H. versicolor* that occurs sympatrically with *H. phaeocrypta* has a faster repetition rate than the race that occurs to the west of it, there are two reasons to doubt that the accelerated repetition results from hybridization-induced reinforcement. First, the contact between these call races of *H. versicolor* (fig. 2) occurs about 100 miles west of the western boundary of *H. phaeocrypta.* To rationalize this discrepancy would require a formerly wider distribution of *H. phaeocrypta* westward or a spread of the fast trilling character beyond the zone of sympatry. Secondly, the duration of the call of *H. versicolor* is longer, and therefore more like that of *H. phaeocrypta,* in the zone of sympatry than in any other area (table 3).

The available analyses of mating call in allopatric and sympatric species pairs of anurans strongly imply that differentiation of mating call to the extent that the differences serve as an important isolation mechanism has occurred under geographic isolation. They also imply equally strongly that in some instances reinforcement of call difference as an isolation mechanism does occur when previously isolated populations come into contact before developing a completely effective set of isolation mechanisms. To summarize, allopatric species pairs show every degree of differentiation in call from essentially none (*Pseudacris streckeri* and *P. ornata*) to strong differentiation (*Hyla femoralis* and *H. arenicolor*). In some species, as in *Hyla versicolor,* the presence of call races suggests that geographic separation has been followed by secondary contact and interbreeding prior to the evolution of an isolation mechanism complex. Some sympatric species show strong differentiation in mating call both inside and outside of the area of contact. These are presumed to have differentiated the call under geographic separation. The only alternative would be the unlikely possibility that differences reinforced in the area of contact had subsequently spread throughout the ranges of the respective species. Other sympatric species show accentuation of differences in the zone of sympatry as an apparent result of natural selection.

LOSS OF CALL

In spite of its functions of aggregating populations for reproduction and of species identification, the call is vestigial or has been lost in some anurans. In the United States, all of the instances of call loss or reduction are found in the far west, where the number of species is relatively few for any genus and the identification function may be relatively unimportant. The large desert toad, *Bufo alvarius,* of the Southwest has a vestigial call and vestigial vocal pouches (Blair and Pettus, 1954). The large size of this toad apparently precludes cross-mating with any sympatric species of

toads. Another species of the same presumed species group, *B. boreas*, apparently lacks a mating call, or at most has a very weak one. A "release note" is used by males of both species, and various reports of a weak mating call in *B. boreas* may refer to this note. In the *Rana boylei* group of the extreme western United States and Mexico, only one (*R. boylei*) of the six species possesses a vocal pouch (Zweifel, 1955). All of these allopatric species are disjunctly distributed except *R. boylei* and *R. muscosa*, which show slight overlap. *R. boylei*, the species which has retained the voice shares practically all of its range with another species, *R. aurora* (Zweifel, *op. cit.*). The four Mexican species, *R. tarahumarae*, *R. moorei*, *R. pueblae*, and *R. pustulosa*, all occur in the range of *R. pipiens*. Another voiceless western anuran, *Ascaphus truei*, is a primitive type with no other representative of its suborder outside of New Zealand.

Inger (1954) has described a remarkable instance of geographic variation in the presence or absence of vocal pouches in *Rhacophorus leucomystax* of the Philippines and adjacent areas. Vocal pouches are present in some areas and absent in others. Three of 37 males from northeastern Borneo had vocal pouches.

NATURAL HYBRIDIZATION

All of the well authenticated reports of natural hybrids between species of U. S. toads that have been published involve crosses between *Bufo woodhousei* and members of the *B. americanus* group (A. P. Blair, 1941, 1942, 1955; Volpe, 1952; Cory and Manion, 1955; Stebbins, 1951; W. F. Blair, 1956a) or *B. valliceps* (Thornton, 1955; Volpe, 1956). As originally held by A. P. Blair (1941) and generally concurred in by the other workers, the disturbance of ecological isolation mechanisms through man's interference with the environment is probably responsible for this hybridization. The untrilled call with broad side-band structure of *B. woodhousei* is very different from the trilled call of *B. valliceps* and the trilled, finely tuned call of the members of the *B. americanus* group. It is evident in these instances of hybridization that call difference alone is not sufficient to prevent some sympatric hybridization when other isolation mechanisms are weakened or eliminated. Sympatric hybrids between *Scaphiopus couchi* and *S. burteri* (Wasserman, 1957) are also attributed to man's disturbance of the landscape. Hybrids are also known between *Scaphiopus hammondi* and *S. bombifrons* (W. F. Blair, 1955c), but the degree of hybridization is unknown.

There is limited information about the calls of species hybrids. The call of a laboratory-produced, field-raised hybrid between *Bufo terrestris* and *B. houstonensis* is intermediate in repetition rate between the calls of the parental species. The former species has a shorter call with a faster repetition rate than the latter one (W. F. Blair, 1956a). The hybrid had an average duration of 8.0 seconds and a repetition rate of 39.0 pulses per second at an air temperature of 23.0°C. Two *B. terrestris*, similarly labora-

tory produced and field raised, and recorded at the same temperature, had average durations of 5.7 and 5.8 seconds and repetition rates of 52.0 and 53.3 pulses per second. By contrast with the intermediacy of the call of hybrids between these closely related species, the call of sterile hybrids between the distantly related *B. woodhousei* and *B. valliceps* are imperfect but tend to resemble the call of the male *valliceps* parent (W. F. Blair, 1956c).

SUMMARY

Analysis of the mating call is useful for the study of evolutionary problems in anuran amphibians because characteristics of the call are chiefly related to the functions of attracting a mate and of species identification in breeding congresses. Use of the sound spectrograph in recent years has made possible the measurement and objective comparison of calls.

Variations in call occur among individuals in local populations and between the calls of the same individual under different conditions. Frequency and repetition rate tend to increase with increased environmental temperature and to decrease with increased body size. The call may vary in relation to sexual excitement. The variation in call of a single *Hyla versicolor* recorded on nine different nights and the variations among 17 *Pseudacris streckeri* recorded from a breeding population on a single night are discussed. Variations not explainable in terms of environmental or ontogenetic factors or degree of sexual excitement are deemed to have a genetic basis.

Patterns of geographic variation in call are discernible in species which have been investigated. In *Pseudacris nigrita,* on present information the call races tend to correspond to subspecies described on morphological bases. In *Hyla versicolor,* the relationships of three call races seem best explained by the hypothesis of secondary interbreeding after Pleistocene separation of three segments of the population.

Each species of anuran has a call that differs from the call of every other species. Groups of species within a genus, however, show basic similarities in call structure, which, with other evidence, are indicative of evolutionary relationship. Species groups in the genera Scaphiopus, Bufo, and Hyla of the United States are suggested on the basis of call structure and other evidence.

Various allopatric and sympatric species pairs are discussed in relation to their bearing on the question of purely geographic speciation versus geographic separation with subsequent reinforcement of isolation mechanisms. Some allopatric species (*Pseudacris streckeri-P. ornata, Scaphiopus bolbrooki-S. burteri*) show little differentiation in call. Others (*Hyla femoralis-H. arenicolor* and various members of the *Bufo americanus* species group) show strong differentiation. Some allopatric species show as much differentiation in call as do sympatric species. Among sympatric species, *Acris gryllus* and *A. crepitans,* along with previously reported species

pairs, show reinforcement of call differences where they occur together. Some sympatric species pairs (*Bufo compactilis-B. cognatus, Hyla cinerea-H. gratiosa, Hyla versicolor-H. phaeocrypta*) have strongly differentiated calls but show no evidence of reinforcement where they occur together. The evidence from the allopatric and sympatric species pairs is interpreted as indicating that speciation has occurred either through the evolution of effective isolation mechanisms under geographical isolation or through reinforcement of partially effective mechanisms after ranges have become sympatric.

The mating call is weak or lacking in some anuran species. All instances of this in the United States are found in far western species of Bufo, Rana and Ascaphus and in which the identification function of call may be relatively unimportant because of the scarcity of species there.

Natural hybridization sometimes occurs between species with well differentiated calls. All well authenticated natural hybrids among U. S. toads involve crosses between *Bufo woodhousei* with representatives of the *B. americanus* group or with *B. valliceps*. This indicates that call differences are not necessarily sufficient to prevent interspecific hybridization when other isolation mechanisms are weakened or eliminated. Hybrids are also known in Scaphiopus.

The calls of hybrids between closely related species are intermediate in character between those of the parental species. The calls of hybrids between two distantly related species (*Bufo woodhousei* and *B. valliceps*) are imperfect and tend to resemble the calls of the male (*B. valliceps*) parent.

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Vol. XCII, No. 862 The American Naturalist January-February, 1958

EARLY NOTICES OF *THE AMERICAN NATURALIST*[1]

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The historical development of the *American Naturalist* and public reaction to this first American journal of natural history as indicated through correspondence has recently been described (Dexter, 1956). In pursuing further studies, the writer has examined the press notices which appeared in the early days of the *Naturalist's* existence. Many of these notices were so uniform in content and wording that it is apparent they were copied from prepared advertizing circulars distributed by the journal itself. Others, however, were original, at least in part, and represented the critical reaction of reviewers and editors. Some of these are quoted here to demonstrate the evaluation of the *American Naturalist* recorded by writers on the staffs of other publications some four to six years after this pioneering journal had been on the market. The first issue was published in March of 1867. After several years of trying experiences the founders (F. W. Putnam, A. S. Packard, Jr., Alpheus Hyatt, and E. S. Morse—curators at the Peabody Academy of Science in Salem) had placed the journal on a firm footing, scientifically, if not financially.

Frederick Putnam, publisher of the journal and director of the museum, wrote in the *First Annual Report of the Peabody Academy of Science* (1869), "All the numbers of the magazine have been noticed and supported in the most cordial manner by the press, and by the scientists and educators of our country, and it has received several notices of praise from European magazines, and distinguished naturalists." Following are excerpts from some of these early notices.

The Household of Brattleboro, Vt., carried this message in May, 1871, "We consider this our best scientific journal, combining in a fit manner the instructive and interesting," while the *Newburyport Herald* of Aug. 4, 1871, said, "—numbering among its contributors some of the best scientific talent in the country." The *Aylmer Enterprise* of Canada stated in July of that year that, "It is beautifully illustrated and well worth the small amount charged for it yearly—$4.00."

Its timeliness was noted by the *Salem Register* of Oct. 12, 1871. "The present number is entitled 'Association Number' and contains a full and complete abstract of all articles read at the recent meeting of the American Association for the Advancement of Science at Indianapolis in the section of Natural History. The issue of this number within a few weeks after the

[1]Acknowledgment is made to Ernest Stanley Dodge, Director of the Peabody Museum of Salem, Massachusetts, for permission to study records on file at that institution and for his encouragement in my study of the Salem zoologists who founded *The American Naturalist.*

adjournment of the session, speaks well for the energy of the editors." Later that year (Dec. 25) the *Salem Register* claimed that, "This work is a credit to our city, not only in its scientific reputation, which is acknowledged by all to be very high and well sustained, but in the typographical execution as well." Two months later this same paper carried the testimony that it is "—truly deserving of the patronage of the Salem public as a native enterprise and one that has received the highest encomiums from scientific societies and individuals, not only of this country but of Europe."

The Gardener's Monthly of Philadelphia expressed in the Nov., 1871, issue the conviction that, "There is no one who wishes to keep pace with the progress of American science but will find an interest in its pages." *The New Albany Daily Standard* of Indiana carried at the same time a notice that, "This is the only periodical of the kind published in this country, and should be patronized by every lover of nature and the sciences."

From *The College Courant* of New Haven, Conn., of Jan. 6, 1872, came the following praise. "The 'excellent American journal,' as Mr. Darwin calls it, enters upon its sixth volume with the January number. We are glad to see that the proprietors have decided to create a department of microscopy for it and to place this department in charge of Dr. R. H. Ward of Troy, an accomplished microscopist. They have thus added to the value of what was before an invaluable periodical."

The Indiana Journal of Medicine for Jan., 1872, exhorted, "Everyone who pretends to be interested in scientific matters should become a regular subscriber to this valuable magazine," while the *New England Postal Record* for the same month stated that, "During the past year, this monthly has given a large amount of useful and reliable information in natural history."

After five years of publication, "The *Naturalist* maintains its high reputation as a popular illustrated Magazine of Natural History," according to the *Newburyport Herald* of April 16, 1872. However, the *New Jerusalem Messenger* that month took exception to the claim of being a "popular" publication with the comment that, "Although the *American Naturalist* can scarcely be called a popular magazine, notwithstanding the claim to that effect on its title page, yet it will doubtless be found acceptable to many general readers." The *Exeter News Letter* of New Hampshire, on the other hand, was convinced that the magazine did serve both technical and non-technical readers. On June 14, 1872, the following appeared, "Better testimony than this journal affords to the vitality of American thought cannot be given. Here the scientific men of the country discuss problems in natural history on their own level and here the unscientific (sic) reader may find the results of their research adapted to his comprehension. This policy makes the magazine at once popular and scientific."

The *New York Evening Mail* for Feb. 6, 1873, became poetic in its appraisal. The reviewer wrote,

"A most respectable, creditable periodical, not half well enough sustained. I hardly know whether to speak of it as:

A gem of purest ray serene
The dark unfathomed caves of Salem bear;

or as:

A rose, born to blush unseen,
And waste its sweetness on the Salem air.

—we may call this excellent but unappreciated monthly both a gem and a rose."

The New England Farmer for May 24, 1873, was one of the most critical in its stand on the *Naturalist.* About this time the famous Marsh-Cope controversies were raging in its pages. Apparently distraught by the language of the participants the reviewer wrote as follows. "We are sorry to see that a couple of 'Professors', Messrs. Marsh and Cope, are allowed space for personal strictures; especially as the *Naturalist* claimed something distinct from the average thought of our age and people, whether expressed in our commercial, literary, or religious papers. It is the mental air that Galileo, Goethe, Newton, Kant, Linnaeus, Cuvier, Rumford, and the scientific lights of our own day have created, and nothing but sound, mental health, a hearty love of truth, and greater happiness will result from breathing such air. Now Messrs. Editors, keep these 'scientific lights' in this healthy air, and do not allow them to sink below the average plane of our 'commercial, literary, or religious papers.' 'Scientific thought' is too dignified for such expressions as 'misrepresentation,' or 'effrontery,' 'criminally,' 'ambiguous' or 'untrue.' By the way, if 'science is the expression of the common sense of all ages,' why is scientific thought something distinct from the average thought of our age and people?"

Probably the concensus of opinion of most reviewers of the day is well summed up by that expressed in the *Maine Farmer* of Dec., 1873, which states, "Few periodicals possess so much real merit, and are of so permanent value as the *American Naturalist* published monthly at Salem, Mass., by the Peabody Academy of Science and edited by Profs. A. S. Packard, Jr. and F. W. Putnam."

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

THE GENE CONTENTS OF OVERLAPPING INVERSIONS

A chromosome inversion has the well known property of allowing the genes within it to recombine effectively only with those in an identical arrangement; as a result, large sections of a chromosome may be inherited as though they were single genes.

When a second inversion arises so that it overlaps one already in existence, there will be three chromosome arrangements (a 'triad'), and crossing-over between certain regions of the inverted arrangements will give rise to gametes with aneuploid chromosome complements. There should thus be selection against one of these inversions. At the same time, recombination between all three sequences, but not two alone, should result in the partial disintegration of the blocks of genes contained within the inverted regions, and there should be selection against the simultaneous occurrence of, not one of the inversions only, but any one of the three arrangements. Using this argument, Wallace (1953) concludes that the evidence shows that the formation of aneuploid gametes is less important in determining inversion frequencies than is the disruption of the blocks of genes within the inversions."

The evidence which Wallace uses is based on the frequencies of inversions in natural populations of Drosophila. Though populations of Chironomus are also extensively polymorphic for inversions, so far (Acton 1957) the inversions have not been of the kind which would allow their frequencies to be used to test the hypothesis to any great extent. I have since come across a striking demonstration of Wallace's conclusion that the integrity of the gene content of an inverted region can only be maintained if two, but not all three members of a triad occur together.

In Europe, chromosome 3 of *Chironomus tentans* has a 'puffed' region near its centre which marks the position of the nucleolus. In southern Canada the banding pattern of the same chromosome is in most respects identical, except that there is no 'puffed' region. There are two inverted banding sequences in Canada, one of which, 3Lc, occupies most of the left-hand arm of the chromosome, and is overlapped by the other, 3Lb, which has a more central position. Thus the three sequences, 3, 3Lb and 3Lc, form one of Wallace's triads, and according to his hypothesis at least one of these sequences should be rare in a given population. As with some populations of Drosophila there are exceptions; at Toronto the frequencies of the three arrangements are 22%, 39% and 39%, though 3 is rare at Ottawa, Montreal and Churchill.

The 'puff', absent from 3Lc, and all the other sequences of chromosome 3, in southern Canada, occurs at Churchill, but with the sequence 3Lc *only*, and, further, outside the inverted region. Normally, the only way such an association could be maintained would be by selection; but the

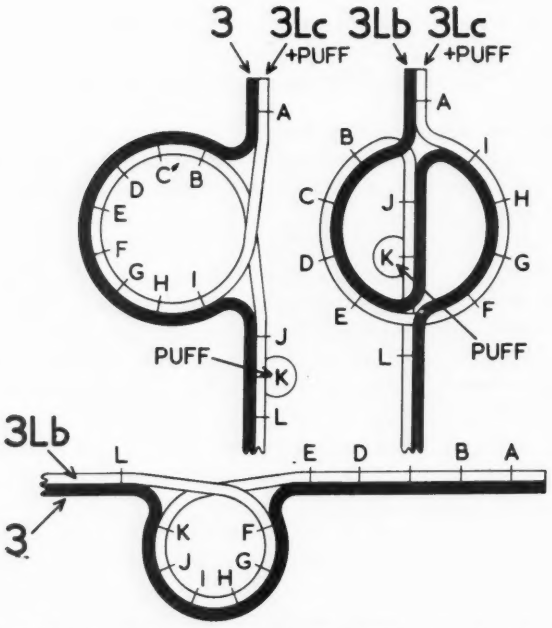

FIGURE 1. 3/3Lc + puff does not occur at Churchill: only when the 'puff' falls outside the inversion loop can it be separated from 3Lc.

fact that the 'puff' falls within the region which is bounded by the other inversion, 3Lb, prevents the separation of the 'puff' from 3Lc by crossing-over, provided the arrangement 3 is absent. The partnership would soon be broken up by recombination, were 3 and 3Lc + puff to occur together.

The sequence 3 occurs in all the localities where the 'puff' is absent from chromosome 3: there was no sequence 3 at Churchill (121 larvae).

I am indebted to the Department of Botany of the University of Toronto for their generous hospitality during my stay in Canada, and to the Royal Society for a grant.

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CROSSING OVER IN HETEROCHROMATIN[1]

One of the genetic characteristics of the centromeric heterochromatin of Drosophila is the low frequency of crossing over in this presumably long region. Mather (1939) proposes, on the basis of his experiments and those of Grüneberg (1933), that this attribute of heterochromatin may be modified by relocation of the material. This conclusion is based on studies of exchange in X-chromosome inversions of *Drosophila melanogaster* which move a large portion of the basal heterochromatin close to the distal end of the chromosome. Crossing over was measured between markers in the distal euchromatin and markers in the originally basal euchromatin, thus spanning the distally located heterochromatin. These authors observed a crossover frequency much greater than the published map distance for the regions involved and interpreted their data to mean that exchanges in centromeric heterochromatin occur more frequently when this material is removed from the centromere. This interpretation, although reasonable, was not necessarily validated by the data, since the markers used did not critically delimit the heterchromatin and thus the high frequency of crossing over observed could be attributed to increased exchange in the adjoining euchromatin. Indeed, Braver (1957) has recently demonstrated just such a high frequency of exchange in the distal euchromatin adjoining the heterochromatin of *In(1)rst*^{3} homozygotes, one of the inversions used by Grüneberg and Mather.

In the *T(3;5)pe*^{m51} reciprocal translocation in *D. virilis*, almost all of the long heterochromatic region at the base of chromosome 5 has been moved to the tip of chromosome 3 (Baker, 1954). Homozygotes for this translocation appear to offer an ideal test of the point in question for these reasons: (1) Crossing over in *virilis* is about four times as frequent as in *melanogaster*. (2) The basal heterochromatin is removed at least 100 crossover units from the centromere. (3) The heterochromatic region is delimited quite specifically by *pe* at the distal end and by *sv* on the proximal side. The gene *pe* is located at the left end of the heterochromatin of chromosome 5, and *sv* has been shown to be less than 0.1 map units from the break point in chromosome 3 of the translocation (as measured in translocation heterozygotes; see Baker, *loc. cit.*).

Females homozygous for the translocation but heteroxygous for eight markers were formed by crossing *T(3;5)pe*^{m51}, *ru st B*^{3} *pe*^{m} *sv* males with *T(3;5)pe*^{m51}, *pe t tb gp*^{2} females. The F_1 females were testcrossed with males of a homozygous *ru st B*^{3} *pe; sv t tb gp*^{2} stock[2]. In figure 1 the genotype of the F_1 females is diagrammed and the frequency of crossing over (based on 1851 offspring) in each of the seven regions is recorded. These

[1]This research was conducted under Atomic Energy Commission Contract AT (11-1)-431.

[2]*ru—ruffles* (5-44.2); *st—scarlet* (5-67.5); *B*^{3}*—Branched* (5-141.0), this allele behaves as a recessive in these stocks; *pe—peach* (5-203.0); *sv—short veins* (3-24.5); *t—telescope* (3-57.5); *tb—tiny bristles* (3-104.0); *gp*^{2}*—gap* (3-119.0).

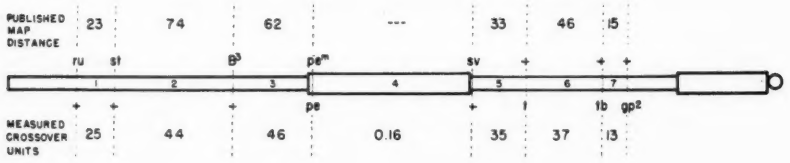

FIGURE 1. Crossover frequencies in the long chromosome of $T(3;5)pe^{m}51$.

frequencies may be compared with the published map distances also given in the figure.

Only three crossovers were recovered in region 4, the distally removed heterochromatin. This frequency is no higher than that observed in translocation heterozygotes (Baker, *loc. cit.*) where, among 2,600 offspring, two crossovers were detected between pe^{m} in the translocated chromosome and the centromere of a normal chromosome 5, and another two were found in the euchromatin between sv^{+} and the break point in the translocated chromosome. Therefore, it might be expected that one or two of the three exchanges detected in region 4 were actually exchanges in the euchromatin between the break point and *sv*. It is important to note that crossing over is normal throughout the rest of the translocated chromosome. (Where map distances are greater than 50 units, the crossover frequency approaches 50%, as expected.) Calculations involving the frequency of recovered double and triple crossovers show no evidence of interference between any of the euchromatic regions.

The evidence seems conclusive that in this case there is little or no exchange in basal heterochromatin when it is removed from the centromere.

The author is grateful to Mrs. Anneke Davis who assisted in these experiments.

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THE MOST WIDELY DISTRIBUTED INVERSION IN THE MOST WIDELY DISTRIBUTED SPECIES OF DROSOPHILA

Brncic and Dobzhansky (1957) reported that *Drosophila funebris* Fabr., 1787, is the only species of the genus Drosophila, known to live in Chile south of the latitude 43°-44° S.. According to Basden (1956), this is also the northernmost member of the genus. Consequently, *D. funebris* represents the most widely distributed species of Drosophila, which extends its area farthest north as well as farthest south. Like other cosmopolitan species, it occurs mostly in close association with man, and has been introduced in Chile probably by human agencies.

The classical works of Dubinin and his school (1937, 1946) have established that *D. funebris* is polymorphic with respect to the gene order in the chromosomes due to the presence of inversions. These authors also detected some correlation between the frequencies of the different chromosomal types and the nature of the ecological niches exploited by the species. A study of the chromosomal structure of the populations of *D. funebris* living in Chile seems therefore of interest.

The material used in the investigations reported in the present note were six genetically very heterogeneous stocks, originating from the following localities of Chile: La Serena, Coquimbo (lat. 29°54' S.), Santiago (33°26' S.), Concepcion (36°50' S.), Centinela, Llanquihue (41°01' S.), Punta Arenas, on the Strait of Magellan (53°10' S.), and Caleta Josefina, Tierra del Fuego (53°40' S.). The last two stocks were maintained for more than a year in the laboratory and were collected in collaboration with Professor Th. Dobzhansky. Thanks to the excellent work of Ann-Margaret Perje (1954), the gene arrangements in the chromosomes could easily be identified.

Five of the stocks were mostly heterozygous for a long inversion in the second chromosome, apparently identical with that found by Perje (1954) in stocks from Lidingo, Sweden, and Paris, France, and according to the author just referred to, corresponding to the C-II-M inversion of Dubinin, Sokolov and Tiniakov (1937), which was found widely distributed in Russian populations (Fig. 1, no. 1). The only Chilean population free from this inversion was that of Concepcion. In the stock from La Serena, some of the flies were heterozygous in addition to the C-II-M inversion for another inversion (Fig. 1, no. 2) located in the middle part of the third chromosome according to the map of Perje. This third autosome corresponds to the fourth one in the nomenclature of Tiniakov (1936). It would not be surprising if the inversion found at La Serena was the IV-1 described by Dubinin and his associates in Russian populations.

The finding that the Chilean populations of *D. funebris* are polymorphic with respect to the gene orders in their chromosomes, and that at least one of the inversions is the same as found in northern Europe, indicates that this genetic arrangement is very old in the evolutionary history of the fly.

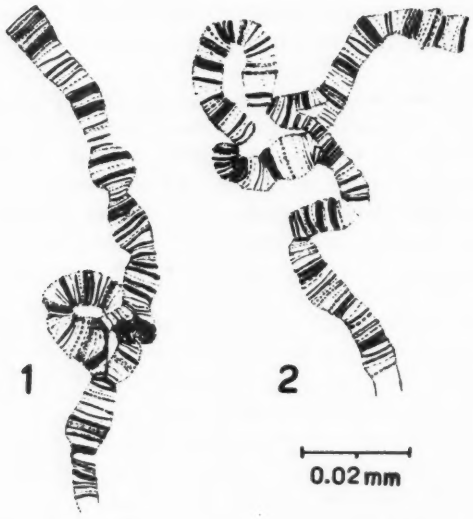

FIGURE 1. 1) Heterozygous inversion found in the middle of the third chromosome. 2) Heterozygous inversion C-II-M in the second chromosome of *D. funebris*.

Such an old and widely distributed kind of polymorphism may be regarded as adaptively important for the species. As *D. funebris* seems to be the most widely distributed species of the genus in the world, inversion C-II-M is actually the most widely distributed inversion ever discovered.

The adaptive nature of the inversions in the genus Drosophila has been demonstiated in many ways. In natural populations of "wild" species, not associated with human habitats, the different gene arrangements usually show quantitative and qualitative geographic variations (Dobzhansky, 1951). "Domestic" species have, on the contrary, chromosomal variants which are widely distributed over the range of the species, and do not normally show differences in the concentration of the inversions from one place to another. Nevertheless, it would be wrong to conclude that the chromosomal variability present in species associated with man is not adaptively important. As Dubinin and Tiniakov (1946) pointed out "human activities create distinct ecological complexes which form an integral part of the environment in which the evolution of the species is being enacted." The observations of these authors have shown that the populations of *D. funebris* that inhabit urban places are distinct with respect to the concentration of inversions from those living in rural localities, and Brncic (1955) has found that in another "domestic" species, *D. immigrans*, there are some relationships between the type of the habitat and the chromosomal polymorphism.

To summarize, though we are far from knowing the ecology of the "domestic" species of Drosophila, and consequently no generalizations can

be made, it seems that "domestic" species are not a special case in which the inversions are adaptively unimportant.

We wish to acknowledge our obligations to the Rockefeller Foundation and to the University of Chile who provided the support for our work.

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ERRATUM

In the article by N. N. Kuznezov, Vol XC, page 350, line 2, for "latitudes 37° and 40° N." read "latitudes 37° and 46° N."